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Research Article

LOCAL ANTIMICROBIAL DELIVERY OF SATRANIDAZOLE LOADED CROSS LINKED PERIODONTAL CHIPS USING BIO DEGRADABLE POLYMERS

SREEJA C NAIR*, ANOOP K R

Department of Pharmaceutics, Amrita School of Pharmacy, Amrita Vishwa Vidyapeetham University, AIMS Health sciences Campus, Kochi, India. Email: sreeju2u@gmail.com

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ABSTRACT

Objective: The goal in using a local nonsurgical controlled intrapocket device for the delivery of an antimicrobial agent is the achievement and maintenance of therapeutic drug concentration for the desired period of time.

Methods: The study was conducted to fabricate a bio erodible delivery system for Satranidazole, dispersed in two different polymers, natural Chitosan and semi synthetic HPMC polymers by simple casting method. The prepared chips were cross linked with glutaraldehyde 2 and 4 %v/v for 1, 2, 3 and 4 hours for extended release.

Results: All chips prepared have good physicochemical properties .Static dissolution studies showed a burst release initially followed by a slow sustained release when cross-linking was attempted. The percentage cumulative drug release was greater in chitosan than HPMC. In vitro release and permeation Kinetics showed zero order profile. The cross linked formulations $C_5$2C3 and $H_5$2C1 are better than others because the extent of release was maintained for 8 and 6 days respectively. Histopathological studies of the periodontal mucosa suggested that the formulations were safe for local anti microbial treatment in to the infected periodontal pocket. The optimized formulation $C_5$2C3 showed highest mucosal permeation of Satranidazole and greater growth inhibition area for Porphyromonas gingivalis. Scanning electron microscopy showed that the upper surface of cross linked chips was smooth.The stability studies did not show any significant changes.

Conclusion: The findings of the results easily predict the fact that chitosan and HPMC can be used to prepare chip utilizing the anti microbial property of Satranidazole for treating periodontitis.

Keywords: Non-surgical periodontal therapy, Satranidazole, Periodontalchips, Chitosan, HPMC

INTRODUCTION

Destructive periodontal disease is a concern because of the potential damage to the dentition and the financial burden of treatment. It is generally agreed that microorganisms residing in periodontal pockets are responsible for periodontitis, but uncertainty exists regarding the exact mechanisms by which periodontal tissues are destroyed. The systemic use of antibiotics raises a number of issues. A prolonged administration increases the risk of problems such as antibiotic resistance[1] and adverse drug reactions like nausea, diarrhoea and pseudo membranous colitis.[2] As a result of these matters, studies focusing on the development of localized drug delivery systems for the release of antibiotics in the periodontal pockets are becoming more frequent. This approach leads to higher concentrations of the drug at the target sites, minimizing the potential systemic side effects. [3]Local delivery of antimicrobials in periodontal pockets may be carried out with fibers, films, microparticles and gels made of biodegradable or non degradable polymers and have been proposed as effective methods to administer antimicrobial agents in periodontal therapy.[4]

Periodontal (gum) diseases, including gingivitis and periodontitis, are chronic bacterial infection that affects the gums and bone supporting the teeth.[5] Satranidazole (SZ) is a 5-nitroimidazole substituted at the 2-position and has been found to be more active against aerobic, microaerophilic, and anaerobic bacteria than MZ. The MIC_{90} of SZ was found to be fourfold lower than MZ against 50 clinical isolates of anaerobes.[6]

The use of natural polymers is valuable based on proven biocompati bility. Chitosan, a natural linear biopolyaminosaccharide, is obtained byalkaline deacetylation of chitin. Properties of chitosan make the polymer suitable for use in biomedical and pharmaceutical form ulations.[7] It has also been used for the encapsulation of drugs.

HPMC is the most important hydrophilic carrier material used for the preparation of oral controlled drug delivery systems. One of its most important characteristics is the high swellability, which has a significant effect on the release kinetics of an incorporated drug .[8] Upon contact with water or biological fluid, the latter diffuses into the device, resulting in polymer chain relaxation with volume expansion.[9] A comparative study of in vitro characteristics of two different polymers based systems was also carried out, to establish the most suitable chip for periodontal application.

MATERIALS AND METHODS

SZ was obtained as a kind gift sample from Alkem, Mumbai, India; Chitosan from CIFT, Kochi,India;HPMC E15 LV from Loba Chemie, Mumbai, India; Propylene glycol from Spectrum Reagents & Chemicals Pvt. Ltd, Kochi, India; Glutaraldehyde from Nice Chemicals Pvt. Ltd, Cochin, India.

Preparation of Periodontal Chips containing Satranidazole

Method used for the preparation of dental chips was solvent casting technique [10] using 1% glacial acetic acid and distilled water as solvent system. Total 72 formulations were prepared. Periodontal chips were prepared by soaking the chitosan in aqueous acetic acid (C2-C5) and HPMC E15LV in distilled water (H2-H5) with propylene glycol as a plasticizer using magnetic stirrer in a closed beaker to get various concentrations of polymers. Satranidazole (1%) was added in required concentrations to each. After complete mixing, 10 ml solution was poured in a clear petridish over aluminium foil placed on a horizontal plane. An inverted funnel was placed over the petridish, with a cotton plug placed into the stem, for slow evaporation of the solvent. The petridish was maintained at 24°C for 24 hours. After complete evaporation of solvent, cast strips were obtained and then cut into pieces of 0.5 x 0.5 cm and each wrapped in aluminium foils and stored in desiccators at room temperature in dark place for further study.[11]

Preparation of Satranidazole loaded Cross-linked Chitosan / HPMC chips

Preparation of chitosan /HPMC chips of different concentrations (3%, 4%, 5%) containing Satranidazole follows the same procedure as above. The chips (C2 to C5 and H2 to H5) were prepared as described were cross-linked by placing in a chromatographic chamber, which was previously saturated with

vapors of 2%v/v / 4%v/v glutaraldehyde solution for extended release. The chips were exposed to vapors in the chromatographic chamber for 1, 2, 3 and 4 hours and then dried. After drying the chips were wrapped in aluminum foil and were placed in desiccators for further study.[12] Typical compositions were showed in Table 1 and 2.

Table I: Composition of various Chitosan formulations (C_2, C3, C4, C5)

Chitosan chip code -				% Drug (SZ)	% Plasticizer (PG)	% of cross linking agent	Duration of Cross linking (hrs)
C2 (2% Chitosan)	C3 (3%)	C4 (4%)	C5 (5%)				
C_2U	C_3U	C_4U	C_5U	1	20	-	-
$C_2$2C1	$C_3$2C1	$C_4$2C1	$C_5$2C1	1	20	2	1
$C_2$2C2	$C_3$2C2	$C_4$2C2	$C_5$2C2	1	20	2	2
$C_2$2C3	$C_3$2C3	$C_4$2C3	$C_5$2C3	1	20	2	3
$C_2$2C4	$C_3$2C4	$C_4$2C4	$C_5$2C4	1	20	2	4
$C_2$4C1	$C_3$4C1	$C_4$4C1	$C_5$4C1	1	20	4	1
$C_2$4C2	$C_3$4C2	$C_4$4C2	$C_5$4C2	1	20	4	2
$C_2$4C3	$C_3$4C3	$C_4$4C3	$C_5$4C3	1	20	4	3
$C_2$4C4	$C_3$4C4	$C_4$4C4	$C_5$4C4	1	20	4	4
-	$C_3$2CG gel	-	-	1	2	2	-

Table II: Composition of Formulation with HPMC (H_2, H_3, H_4, H_5)

HPMC chip code -				% Drug (SZ)	% Plasticizer (PG)	% of cross linking agent	Duration of cross linking (hrs)
H2 (2% HPMC)	H3 (3%)	H4 (4%)	H5 (5%)				
H_2U	H_3U	H_4U	H_5U	1	20	-	-
$H_2$2C1	$H_3$2C1	$H_4$2C1	$H_5$2C1	1	20	2	1
$H_2$2C2	$H_3$2C2	$H_4$2C2	$H_5$2C2	1	20	2	2
$H_2$2C3	$H_3$2C3	$H_4$2C3	$H_5$2C3	1	20	2	3
$H_2$2C4	$H_3$2C4	$H_4$2C4	$H_5$2C4	1	20	2	4
$H_2$4C1	$H_3$4C1	$H_4$4C1	$H_5$4C1	1	20	4	1
$H_2$4C2	$H_3$4C2	$H_4$4C2	$H_5$4C2	1	20	4	2
$H_2$4C3	$H_3$4C3	$H_4$4C3	$H_5$4C3	1	20	4	3
H4C4	$H_3$4C4	$H_4$4C4	$H_5$4C4	1	20	4	4

Preparation of Satranidazole loaded cross linked in situ periodontal gel ($C_3$2CG)

Solution of 3 % chitosan was prepared by dissolving chitosan in 1% acetic acid solution. To this, 2 % propylene glycol was added as a plasticizer. The 1% SZ drug was then mixed with the chitosan solution and was allowed to cross linked with 2% aqueous solution of glutaraldehyde. The mixture was stirred for 30 min at room temperature .The above injectable solution was allowed to keep in room temperature over night to remove bubble.[13] Typical composition is showed in Table 1.

Evaluation of satranidazole loaded chips

The compatibility studies for drug and polymer were conducted by using FTIR spectroscopy.

Physicochemical properties

Physicochemical properties such as thickness, content uniformity, weight variation, folding endurance,surface PH, swelling index, % moisture loss, mass balance studies, in vitro drug release studies, in vitro permeation studies, SEM, mucosa deposition studies, histopathological studies, stability studies and microbiological evaluations were performed on prepared chips.[14,15]

Thickness

The thickness of polymer chips (4×4cm) was determined by using digital screw gauge.

Weight uniformity

Twenty chips of same size (7×2mm) were weighed on electronic balance and average weight was calculated.

Folding endurance Studies

The folding endurance was determined by repeatedly folding one chip at the same place till it broke or folded up to 300 times which is considered satisfactory to reveal good film properties. The number of times the chip could be folded at the same place without breaking gives the value of the folding endurance.

Surface pH

The surface pH of the chips 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 chip 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 1.0 min. The average of three determinations for each formulation is taken.

% Moisture loss studies

The percentage moisture loss study was carried out as per the literature. The chips of known weight and of predetermined size (2 x 2) were placed in a desiccator (containing anhydrous calcium chloride) for three days. The chips were removed and re-weighed, and the percentage moisture loss was calculated as per the formula.

$$\text{Percentage moisture loss} = \frac{(IW - FW)}{IW} \times 100$$

Where IW = Initial weight, FW = Final weight.

Measurement of Swelling Index

The studies for Swelling Index of the chips were conducted in simulated salivary fluid of pH 6.6. The chip sample (surface area: 1.75 cm) was weighed and placed in a pre weighed stainless steel wire sieve of approximately 800 µm mesh. The mesh containing chip 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 chip was determined at each time interval until a constant weight was observed. The degree of swelling was calculated using the formula:

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

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

Drug content uniformity

The drug loaded chitosan chips of known weight of 7X2 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 319 nm. In case of HPMC chip, distilled water was used to dissolve the chip. The polymer solution without drug serves as a blank.

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 know weight and dimension (7×2 mm) of different formulations 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 1 ml was collected on each day and replaced with a fresh 1ml phosphate buffer pH6.6. The concentration of drug in the buffer was measured at 319 nm.The same procedure was continued until no more drug release take place or the chip completely disintegrated. The amount of drug released was calculated and the percentage drug released was plotted against time.[14]

Mass Balance Study

Following the in-vitro release studies (static), the test chips were further analyzed for the drug content left in the chips. Each chitosan chip was dissolved in acetic acid 1% (v/v) and diluted suitably . The HPMC chip was dissolved in distilled water. The absorbance was measured at 319 nm. 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.

***In vitro* Permeation Studies of Satranidazole loaded chips**

For the penetration 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 for approximately for one hour in receptor buffer to gain the lost elasticity. Afterwards the periodontal epithelial mucosa was packed in aluminum foil and stored in a polyethylene bag at −2 °C.[16-18]

A 1 cm^2 chip 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 319nm. 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. Similarly, the permeation studies for all the formulation and for the drug solution, which is, used the control.The cumulative amount of drug permeated per cm^2 was plotted against time, from the slope of the linear portion steady state flux (J_{ss}) can be calculated.[19]

In vitro diffusion study of Satranidazole cross linked in situ gel

A cellophane membrane (cut to suitable size) boiled in distilled water for 1 hour, soaked in absolute alcohol for half an hour and stored in phosphate buffer pH 6.6 for 24 hours before use.. A glass cylinder with both ends open, 10 cm height, 3.7 cm outer diameter and 3.1 cm inner diameter cellophane. Membrane was tied to one end of donor compartment. Gel was accurately weighed, was taken in one cell (donor compartment) and the cell was immersed in a beaker containing 30 ml of phosphate buffer (receptor compartment) of pH 6.6 were used for study. The cell was immersed to a depth of 1cm below the surface of phosphate buffer in the receptor compartment, and temperature maintained at 37±1°C throughout the study.[20] A static dissolution set up was created. Aliquots of 5ml were withdrawn periodically at intervals of 1 day for a period of 5 days and each time equal volume was replaced with fresh phosphate buffer previously heated to 37±1°C. The amount of drug release was estimated using UV spectrophotometer at 319 nm against blank [21].Similarly the in vitro release of control solutions were also performed (PBS PH 6.6 drug solution)for comparison.

In vitro permeation study of Satranidazole cross linked in situ gel

Periodontal mucosal permeation studies of the prepared gel, were carried out using Franz diffusion cell. The epithelial mucosa was fixed onto the Franz diffusion cell. Accurately weighed 1 ml gel was spread uniformly on an area of 1cm^2 of the mucosa, previously fixed in between the donor and receptor compartment of the Franz diffusion cell. The receptor compartment contained 15 ml of phosphate buffer, pH 6.6. The temperature of the elution medium was thermostatically controlled at 37 ± 1^0c by a surrounding water jacket, and the medium was kept as a static model throughout the study. Aliquots of 1 ml withdrawn at predetermined intervals for 48 h, and an equal volume of pre-warmed buffer, was replaced. The samples were analyzed, after appropriate dilution, for SZ content spectrophotometrically at 319 nm against appropriate blank. Similar experiments were conducted with control (PBS PH 6.6 drug solution) also for comparison. [22,23]

Mucosa Deposition Studies

After performing the above-mentioned in vitro permeation study for 48 hr, the donor compartment was washed five times with methanol. The epithelial mucosa was extracted with methanol as a receptor solution for a further period of 12 hours and the amount of the drug was determined by spectrophotometricaly at 319 nm. During this stage, methanolic receptor solution will diffuse into the mucosa, releasing both bound and free form of drug.

Morphological studies

The prepared periodontal mucosa was treated with optimized chip formulation. The epithelial mucosa was washed thoroughly and kept in 10% buffered formalin solution. The specimens were cut into section vertically. Each section was dehydrated using ethanol, embedded in paraffin for fixing and stained with hematoxylin and eosin. These samples were then observed under light microscope and compared with control sample. The changes in the mucosa after treatment were evaluated to detect any damage to the tissue during in vitro permeation by a pathologist blinded to the study.[24]

Scanning electron microscopy (SEM)

The morphology and surface topography of the optimized chip 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^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 and photomicrographs of suitable magnifications were obtained.[14]

***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 optimized film $C_5$2C3, in stu gel formulation (C32CG) and the standard drug solution sample was 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 sample at different concentration (5µl, 10µl, 15µl and 20µl) were added in

each well separately. The anti bacterial activity of the blank film was also performed.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 were performed. [25-27]

Stability studies

The optimized chip formulation $C_5$2C3 was kept for stability studies for 45 days at room temperature (30 ± 2°C) , a refrigerator temperature (4 ± 2°C) and oven temperature (45 ± 2°C) to determine physical and chemical stabilities. The formulation was evaluated visually and for drug content and % cumulative drug release after 7, 15, 30 and 45 days.[14]

RESULT

The procedure followed, results in the fabrication of uniform Satranidazole-polymeric periodontal chips. Compatibility studies showed no interaction between the drug and polymer, by FTIR studies (Figure 1 – 3).The physicochemical evaluation data reveals that the thickness of dental chips increases as the concentration of the polymer increases from 0.05 to 0.25 mm for C2 to C5 and from 0.04 to 0.16 mm for H2 to H5 . The average weight of chips varies from 1.3 mg to 2 mg for C2 to C5 and from 1.2 mg to 1.9 mg for H2 to H5. The percentage moisture loss decreased from 12 to 7% for C2 to C5 and 13 to 9% for H2 to H5. Surface pH for all formulations of chitosan (C2 to C5) ranges from 5.6 to 6.4 and HPMC (H2 to H5) from 6.1 to 6.4. The folding endurance was found to be 50 times greater for formulations containing chitosan as compared to HPMC. Swelling index value increases as the concentration of polymer increases and was greater for HPMC (from 30 to 38% for H2 to H5) than chitosan (from 20 to 34% for C2 to C5%) formulations. All the formulations exhibited good drug content uniformity and was found to be lesser for strips containing HPMC and was greater for chitosan formulations shown in Figure 3. The residual drug content in the chip after *invitro* release was determined and it did not differ from the experimental drug content by more than 3 %. . In vitro release and permeation Kinetics showed zero order profile. The cumulative percentage amount of drug release was found to be 98.0620± 0.7502 , 94.7607 ± 0.2810, 95.1615±1.2162, 94.1041±1.0311, 93.848±0.2599 and 55.58±1.087% respectively for $C_5$2C3, $H_5$2C1, $C_3$2CG, C5U, H5U formulations and control at the end of 192 hours, 144 hours, 120 hours, 96 hours, 72 hours and 24 hours respectively showed graphically in Figure 5. The rank order of drug permeation from selected formulations was found to be $C_5$2C3 > $H_5$2C1 > $C_3$2CG > Control (Figure 6). The ex-vivo permeation studies depicted in Figure 7 concluded that optimized chip formulation was enhanced also 2 times than the *insitu* gel formulation. Among different formulations, $C_5$2C3 optimized chip showed highest mucosal deposition of Satranidazole and the higher concentration of drug get deposited in the mucosa were shown graphically in Figure 8 . The optimized formulation $C_5$2C3 showed greater growth inhibition area for Porphyromonas gingivalis than gel $C_3$2CG (Figure 9).The statistical ANOVA Table performed for antibacterial activity tabulated in Table III confirmed that since the P-value of the F-test was less than 0.05, there was a statistically significant difference between the means of the different variables at the 95.0% confidence level. The microscopic observations indicated in Figure 10 proved that the optimized formulation (C52C3) has no significant effect on the microscopic structure of mucosa. The controlled release rate of formulation(C52C3) was correlated with nonporous structure and high compactness of cross linked chip as seen from the SEM image (Figure 11). Scanning electron microscopy (SEM) showed differences in the surfaces of the chips. From the stability studies it was confirmed that the optimized chip formulation of Satranidazole (Figure 12) remained stable at room temperature (30 ± 2°C) and at refrigerator temperature (4 ± 2°C).

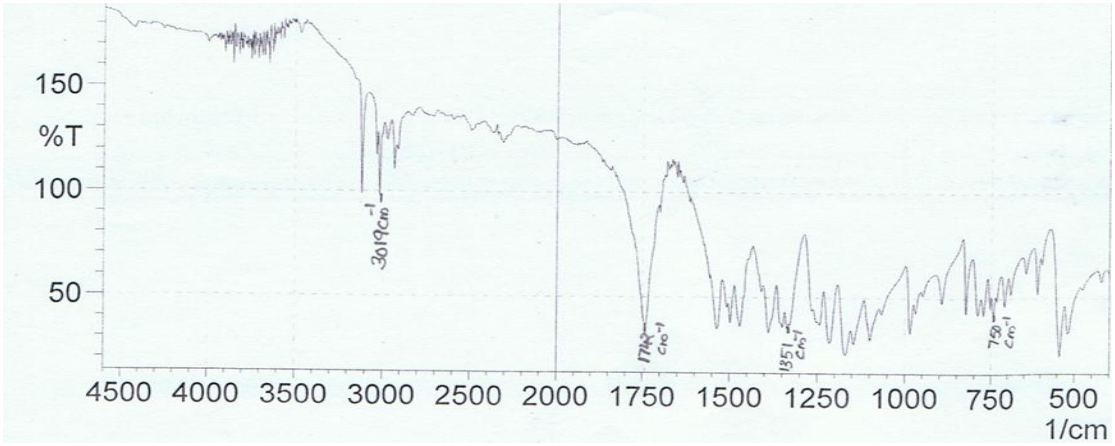

Fig. 1: FTIR of Satranidazole

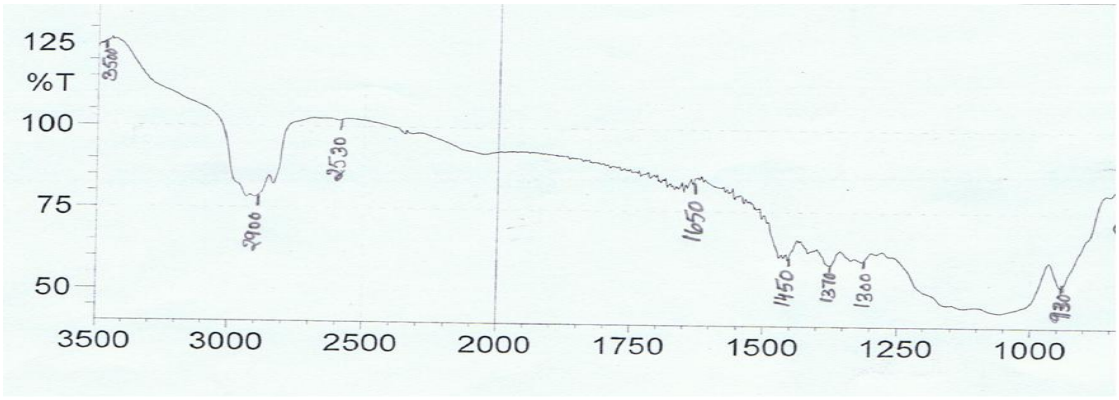

Fig. 2: FTIR of Drug Chitosan Mixture

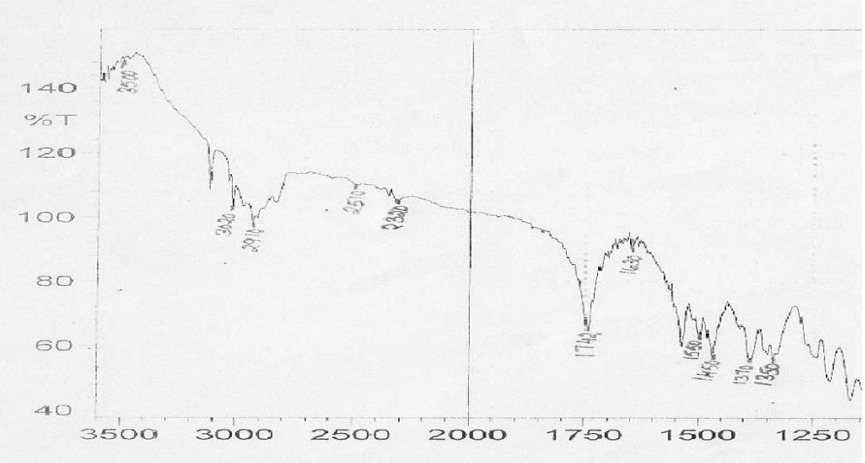

Fig. 3: FTIR of Drug HPMC E15LV Mixture

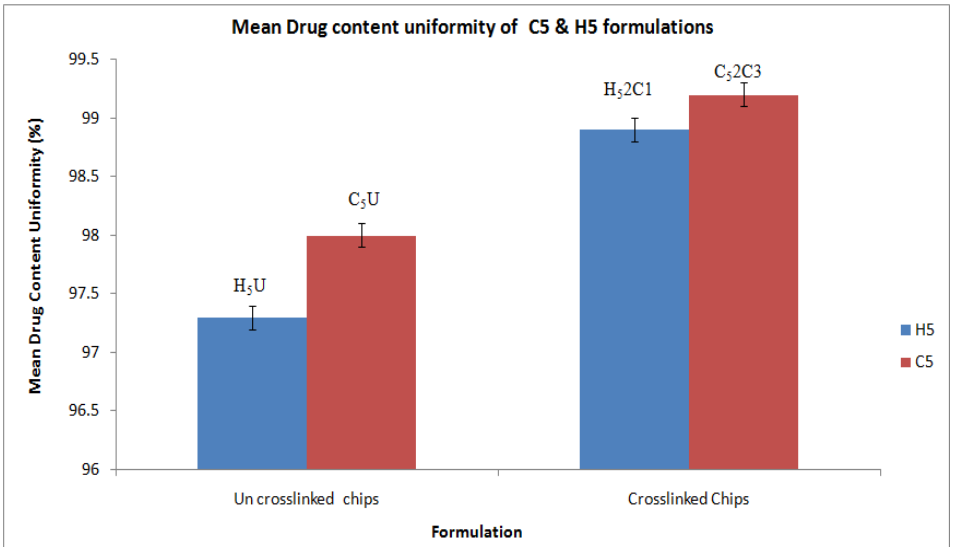

Fig. 4: Mean Drug content uniformity of C5 & H5 formulations

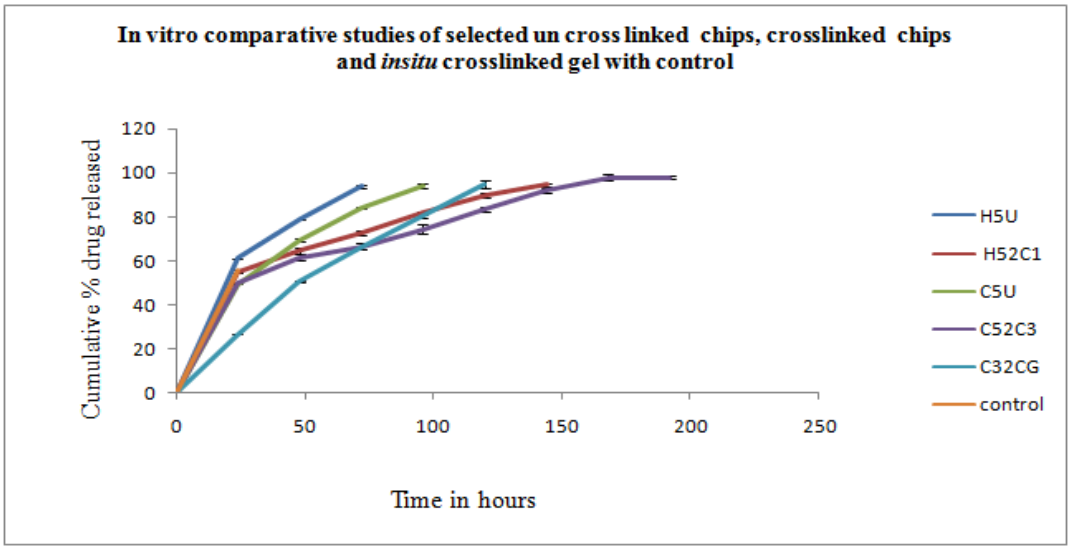

Fig. 5: *In vitro* release comparative studies of selected formulations with control

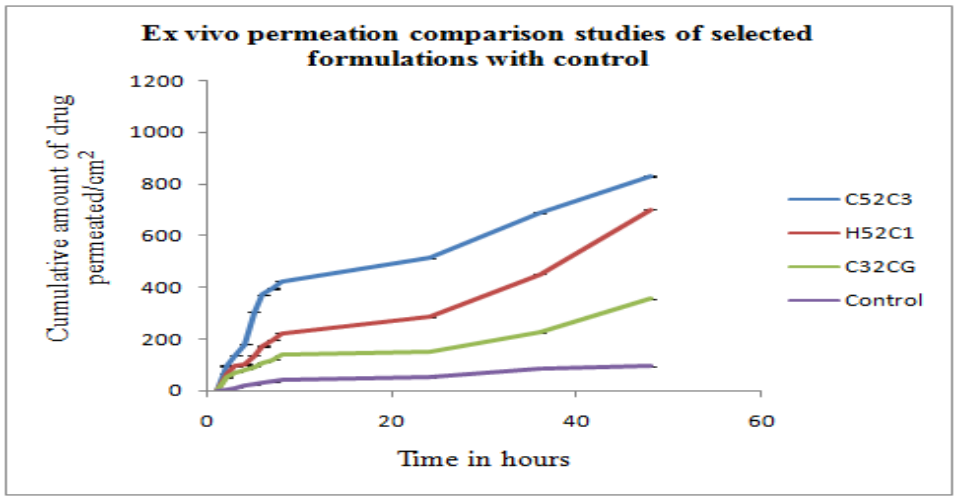

Fig. 6: Ex vivo permeation comparison studies of selected formulations with control

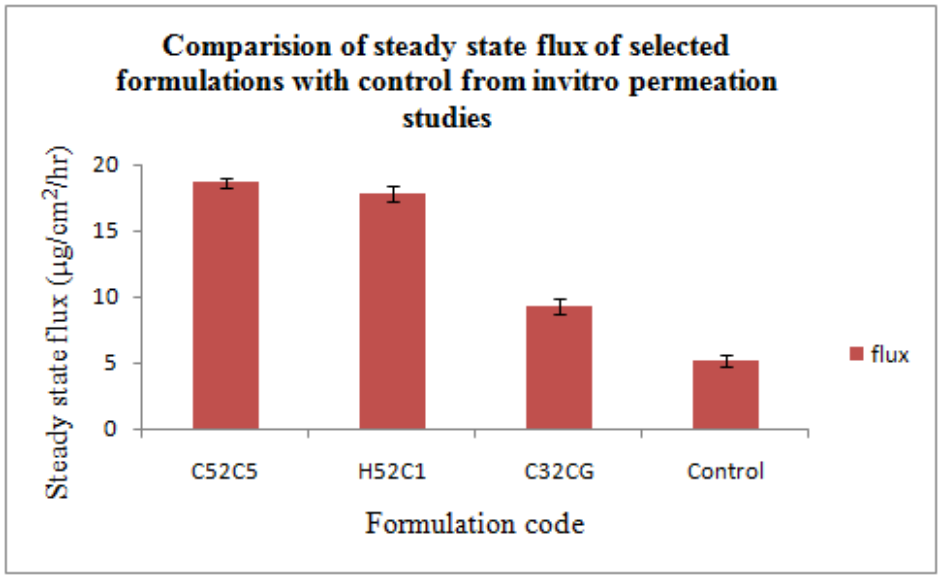

Fig. 7: Comparison of steady state flux of selected formulations through bovine periodontal mucosa

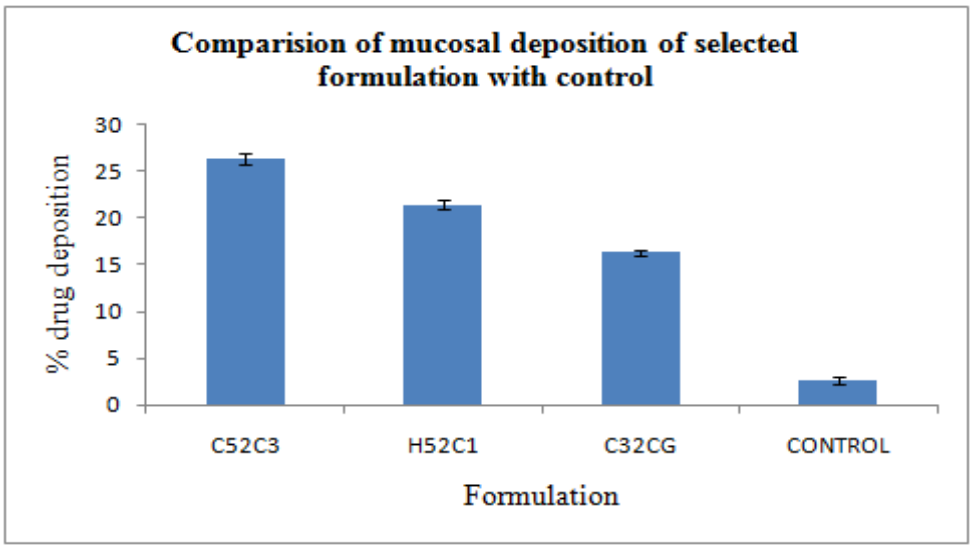

Fig. 8: Comparison of mucosal deposition of selected formulations

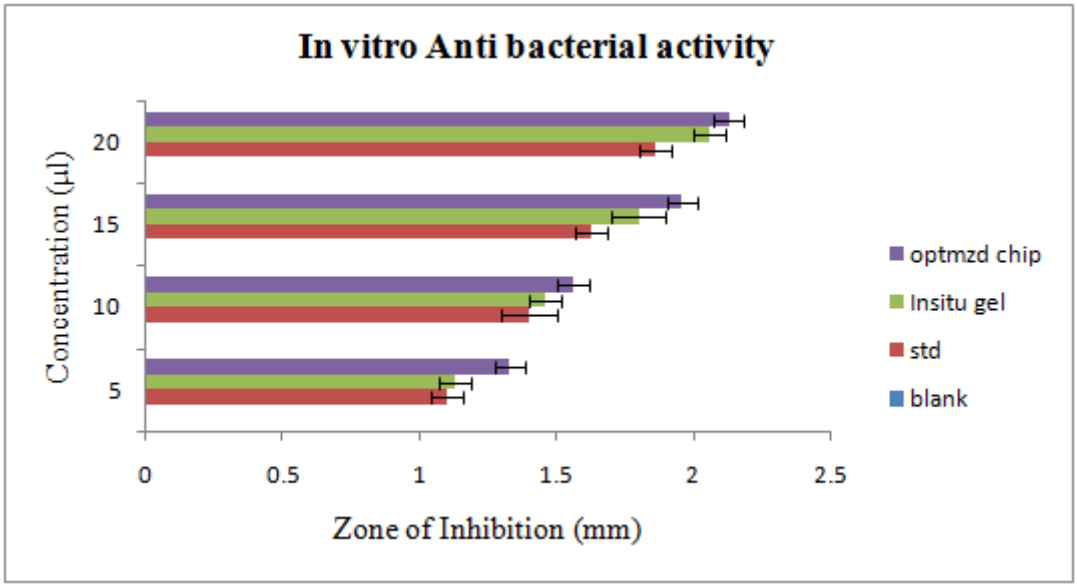

Fig. 9: *In vitro* Anti bacterial activity

Table III: Statistical analysis of Antibacterial activity of different concentration of various formulations table by ANOVA method

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	7.97972	3	2.65991	26.40	0.0000
Within groups	1.20885	12	0.100737		
Total (Corr.)	9.18857	15			

Optimized Chip treated mucosa

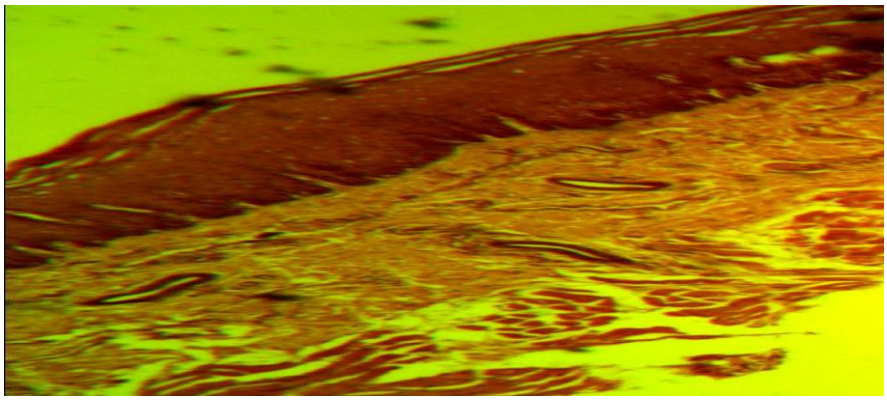

Fig. 10: Photographs of histology of mucosa treated with optimized chip

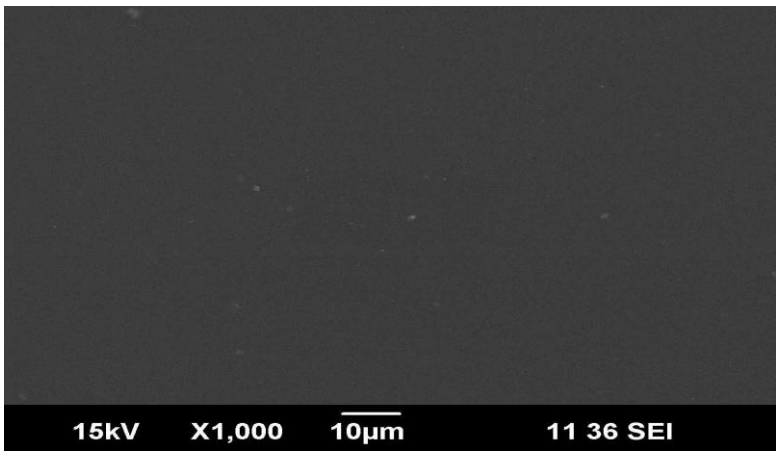

Fig. 11: Scanning electron microscopy of Optimized cross linked chip formulation (C52C3)

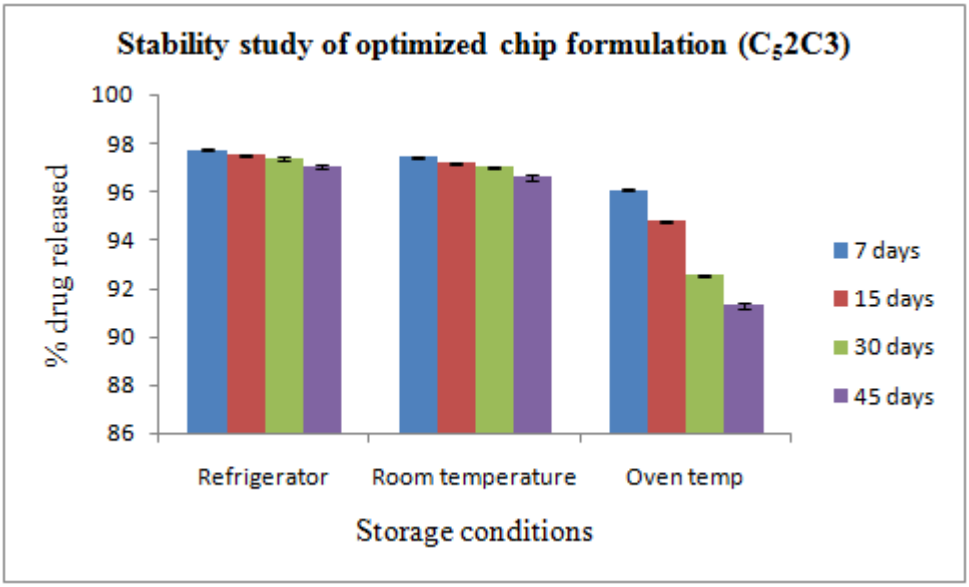

Fig. 12: Stability Study of Optimized chip formulation (C52C3)

DISCUSSION

In the present investigation, Chitosan chips (C_2 – C_5) using 1% v/v acetic acid in water and Hydroxy Propyl Methyl Cellulose (HPMC E 15 LV) chips (H_2-H_5) using distilled water containing drug Satranidazole(1%) were prepared by solvent casting method with the incorporation of propylene glycol as a plasticizer . The prepared chips were further cross-linked with gluteraldehyde 2 and 4 %v/v for 1,2,3 and 4 hours which was aimed to extend and control the drug release for more number of days. The drug loaded chips were flexible and the physicochemical evaluation parameters were found to be satisfactory. The average weight and thickness of chips increase as the polymer concentration increases.The surface pH of the all the formulations was very close to the neutral pH, indicated negligible irritation to the mucosal membrane. The % moisture loss decreases as the polymer concentration increases and the uncrosslinked chips showed greater moisture loss than the cross-linked chips due to the greater compactness and hence lower porosity of the cross-linked chips. All f formulations exhibited good folding endurance exceeding 300, indicating that they are tough and flexible.The chitosan formulation showed 50 times greater folding endurance as compared to hydrophilic HPMC chips due to hydrophobic characteristics of chitosan..The drug content studies showed uniform and homogeneous distribution of drug into the chips. Since the chip remains immobile in the periodontal pocket, a static dissolution model was adopted for the dissolution studies. In vitro release studies performed using PBS 6.6 released the drug in a biphasic manner and showed an initial burst release by more than 40%, which is expected to kill most of the periodontal organism, followed by controlled release for about 3 to 8 days for different formulations, which was above the minimum inhibitory concentration of Satranidazole. The percentage cumulative drug release is greater in chitosan than HPMC E15LV, as hydrophilic polymers are easily degraded by the saliva and gingival crevicular fluid (GCF) and it may also be due to formation of pores which result in drug entrapment to a larger extent and amount. The above study showed that the cross linked formulation $C_5$2C3 of chitosan and $H_5$2C1 of HPMC are better formulations for delivering the drug into the periodontal pockets for 8 days and 6 days respectively with maximum drug release. Of all the 72 formulations, polymer chitosan treated $C_5$2C3 formulation was the optimized formulation , better suited for periodontitis compared to HPMC released the drug for 8 days with cumulative % drug release of 98.0620%. The release kinetics of drug was found to be zero order. Highuchi's diffusion model gave a better and best fit of release data indicating diffusion dominating. The insitu gel formulation of Satranidazole ($C_3$2CG) having controlled release characteristics , releases the drug for a period of 5 days with cumulative % release of 95.1615%. Ex vivo permeation studies of Satranidazole from both the chip and *in situ* gel formulations indicated slow and sustained permeation of the drug for 1 to 48 hrs Permeation study revealed that the permeation flux of optimized formulation was greater compared to gel formulation. The formulation $C_5$2C3 showed greater growth inhibition area against porphyromonas gingivalis than insitu gel. Histopathological study of the periodontal mucosa after permeation study suggested that the the optimized chip formulation were safe for local anti microbial treatment in to the infected periodontal pocket. Scanning electron microscopy (SEM) showed that the upper surface of cross linked chip was smooth and was devoid of pores indicated the the drug release were of controlled manner. The drug remained intact and stable in the periodontal chips during storage, with no significant chemical interaction between the drug and the excipients. These findings suggested that the developed formulation was a viable alternative to conventional drug to cure periodontitis.

CONCLUSION

In the present study it can be concluded that the Satranidazole loaded cross linked chips can be used for local sustained drug delivery system into the infected periodontal pockets using different polymers. The natural polymer chitosan is better suited for periodontitis compared to HPMC as it can be homed into the periodontal pocket by releasing the drug for 8 days having major advantages as the drug is concentrated at its site of action, which reduces possible side effects and the sustained release action allows the pharmacological effects for the extended period of time at the site of infection by increasing the patient compliance and decreasing the systemic side effects of conventional therapy. The cross linking had a definite influence on the release rate of the drug. Further, detailed investigation is required to establish in vivo efficiency of the cross linked chips.

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