



Research Article

CROSS LINKED CHITOSAN *IN SITU* GEL OF SATRANIDAZOLE FOR INTRA PERIODONTAL DRUG DELIVERY

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ABSTRACT

This paper introduces a novel type of injectable temperature-sensitive chitosan polymer based *in situ* gel for the local delivery of Satranidazole into the infected periodontal pocket. The thermo gelling polymer, chitosan with different concentrations has been used for formulation of *in situ* gel of Satranidazole with 10 % propylene glycol as plasticizer and was allowed to cross linked with 1 % solution of glutaraldehyde for extended release. The FTIR studies and the XRD studies confirmed the absence of chemical interaction between the drug and the polymer. The developed formulations were evaluated for various parameters like surface pH, gelation temperature, drug content, spreadability, viscosity, *in vitro* drug release and *in vitro* antibacterial activity, SEM and stability studies. The optimized formulation G6 in terms of cumulative percent drug release along with zero order kinetic mechanism with 98.6 % drug release for 5 days and fulfilled many requirements of once a week delivery system. Throughout the permeation study, the average permeation rate for *in situ* gel was found to be above the minimum inhibitory concentration of Satranidazole indicating the suitability of formulating Satranidazole as a controlled release local delivery *in situ* gel for longer periods of time. Histopathological and microscopic study of the periodontal mucosa after permeation study suggested that the gel formulations were safe for local anti microbial treatment in to the infected periodontal pocket. The *in vitro* antibacterial activity demonstrated a significant antibacterial profile of the *in situ* gel G6 formulation against Porphyromonas gingivalis. The SEM of *in situ* gel suggesting that the drug(s) were dispersed rather than dissolved in the polymer matrix. The stability studies confirmed that the *in situ* gel formulation of Satranidazole remained stable at room temperature (30 ± 2°C) and refrigerator temperature (4 ± 2°C).

Keyword: Injectable system, Periodontal pocket, local delivery, bio adhesive polymer.

INTRODUCTION

The bacteria that cause periodontal gum disease will be eliminated or inhibited through the use of antimicrobial therapy. The aim of current periodontal therapy is to remove the bacterial deposits from the tooth surface and to shift the pathogenic micro biota to one compatible with periodontal health. The periodontal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption. The periodontal cavity offers a number of unique advantages such as easy accessibility, good permeability especially for lipophilic, low molecular weight drugs, avoidance of harsh environmental conditions and hepatic first pass metabolism. The use of bio adhesive polymers can lengthen the residence time and enhance bioavailability of drugs delivered to the periodontal cavity.¹ Success of any drug system designed to target periodontal infection depends upon its ability to deliver the anti microbial agent to the base of pocket at a bacteriostatic or bactericidal concentration. It must also facilitate retention of medicament long enough to ensure an efficacious result.² Local applications (as mouth rinse, gels, tooth paste etc.) control only supra gingival microbial plaque or periodontal disease involving pocket formation and also requires high initial concentrations and multiple applications in order to provide sustained effectiveness.³ Local application of antibiotics has been achieved either by sub gingival irrigation or by incorporating the drug into different devices for insertion into periodontal pockets⁴. Sub gingival irrigation of antimicrobial involves local drug delivery but not controlled release.⁵ Injectable systems are particularly attractive for the delivery

of drug into the periodontal pocket. This application can be easily and rapidly carried out, without pain and using a syringe.⁶ The chitosan has favourable biological properties such as biodegradability and biocompatibility which has attracted a lot of attention in the pharmaceutical and medical fields and an attractive material for multiple applications.⁷ Blending of chitosan with other polymers and cross linking are both convenient and effective methods of improving the physical and mechanical properties of chitosan for practical applications. Immunization studies carried out on rats using glutaraldehyde cross linked chitosan spheres showed promising tolerance by the living tissues of the rat muscles.⁸ The drug Satranidazole exhibits its anti bacterial effect by extensive DNA damage characterized by helix destabilization and strand breakage. 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). It is a highly potent; well tolerated. It is rapidly absorbed and exhibits higher plasma and liver concentration than metronidazole.⁹ So in the present research work, an attempt has made to formulate *in situ* gel of Satranidazole using a natural polymer, chitosan for the effective management of periodontitis with local delivery into the infected periodontal pockets.

MATERIALS AND METHODS

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

The drug was characterized for various pre formulation studies.

Pre formulation Studies

Drug solubility studies

The solubility of Satranidazole in a variety of solvents was carried. Excess amount of drug (100 mg) was added to 10 mL of various solvents. The dispersions were shaken in a thermostatically controlled water bath shaker at $37 \pm 0.5^\circ\text{C}$ until equilibrium (48 h). Afterward, samples were withdrawn, filtered through a $0.45 \mu\text{m}$ membrane filter and suitably diluted. Drug concentration was analyzed and the solubility of the drug in different solvents after suitable dilution, absorbance of solution was measured at 319 nm by using UV visible spectrophotometer.¹⁰

Fourier transform infrared spectroscopy (FT-IR)

FTIR spectra of pure drug, chitosan and the physical mixture (SZ + Chitosan) were obtained using KBr pellet method (applying 6000 kg/cm^2). Spectral measurements were obtained by powder diffuse reflectance on a FTIR spectrophotometer (Shimadzu, USA). Each spectrum was recorded in the frequency range of $4000\text{--}450 \text{ cm}^{-1}$.

X-ray diffractometry

The X-ray diffractograms of Satranidazole, Chitosan and the Physical mixture (SZ + Chitosan) were recorded by using a Shimadzu XD-5 Diffractometer with tube anode Cu over the interval $4\text{--}40^\circ/2\theta$.¹¹

Preparation of *in situ* Gels

In the present study six batches of Satranidazole cross linked *in situ* gels were prepared using natural biodegradable polymer, Chitosan in variable concentrations using glutaraldehyde as a cross linking agent. First, appropriate concentrations of polymer solutions (0.5, 1, 1.5, 2, 2.5 and 3 %) were prepared by dissolving chitosan in 1 % dil acetic acid into a glass vial. The vial was placed on a continuous shaker overnight at room temperature to completely dissolve the polymer. Then, 10 % v/v propylene glycol as plasticizer was added into the polymer solution and mixed together. 1 % w/w of drug Satranidazole was added to the drug-free formulation. All formulations were clear, homogeneous solutions at room temperature. To get cross linked *in situ* gel of chitosan, preparation containing SZ were allowed to cross linked with 1 % aqueous solution of glutaraldehyde. The mixture was stirred for 30 min at room temperature until it became increasingly viscous. The viscous solution was left at room temperature to remove bubble.¹² Typical composition showed in Table 1.

Evaluation of *in situ* Gels

The developed formulations were evaluated for various parameters like pH, gelation temperature, drug content, bio adhesive strength, viscosity, *in vitro* drug release and permeation studies, mucosa deposition studies, histopathology, *in vitro* antibacterial activities and finally stability studies.^{13–19}

Measurement of Gelation Temperature

A 2 ml aliquot of gel was transferred to a test tube, immersed in a water bath. The temperature of water bath was increased slowly and left to equilibrate for 5 minutes at each new setting. The sample was then examined for gelation, which

was said to have occurred when the meniscus would no longer moves upon tilting through 90° .

Surface pH

An acidic or alkaline formulation is bound to cause irritation on mucosal membrane and hence this parameter assumes significance while developing a mucoadhesive formulation. The surface pH was determined by the method similar to Bottenburg *et al.* A digital glass electrode pH meter was used for this purpose. pH was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 minute.

Viscosity Study

Viscosity of gels was studied on Brookfield viscometer by using spindle number 3 at 60 revolution per minute (RPMS) at constant temperature.

Spreadability

The spreadability of the gel formulations was determined 48h after preparation, by measuring the spreading diameter of 1 g of the gel between two glass plates after 1 minute. The mass of the upper plate was standardized at 125 g. The spreadability was calculated by using the formula $S = m \cdot l/t$, where S is spreadability, m is the weight tied to the upper slide, l is the length of the glass slide, and t is the time taken. Homogeneity of gel formulation was tested by visual observations.

Drug Content

1 ml of formulation was taken in 10 ml volumetric flask, diluted with 1 % dil. acetic acid and volume adjusted to 10 ml. Finally the absorbance of prepared solution was measured at 319 nm by using UV visible spectrophotometer (Shimadzu UV). Concentrations of drug were calculated from the standard calibration curve prepared in 1 % dilute acetic acid.

Viscosity Measurement

The viscosity of the prepared gels was measured using a Brookfield at a controlled temperature of $25 \pm 2^\circ$ at 50 rpm.

In vitro Drug Release Studies

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 1 cm below the surface of phosphate buffer in the receptor compartment, and temperature maintained at $37 \pm 1^\circ\text{C}$ throughout the study. A static dissolution set up was created. Aliquots of 5 ml 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 $37 \pm 1^\circ\text{C}$. The amount of drug release was estimated using UV spectrophotometer at 319 nm against blank. Similarly the *in vitro* release of control solutions were also performed (PBS PH 6.6 drug solution).^{20–23}

In vitro Permeation Study

Fresh periodontal tissue was removed from bovine periodontal cavity obtained from local slaughter house. Periodontal mucosal permeation studies of the prepared gel were carried out using Franz diffusion cell. Pre-treated epithelial mucosa was fixed onto the Franz diffusion cell. Accurately weighed 1 ml gel was spread uniformly on an area of 1 cm² 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°C 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. The cumulative drug permeated per unit area was plotted against the time. The slope of the plot was noted and compared. Similar experiments were conducted with control (PBS PH 6.6 drug solution) also. Blanks were run for each set as described above, using placebo gel.²⁴

Mucosa Deposition Studies

Perform the *in vitro* permeation study for 48 h, and then the donor compartment was washed five times with methanol. The periodontal 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 spectrophotometrically at 319 nm. During this stage, methanolic receptor solution will diffuse into the mucosa, releasing both bound and free form of drug and are estimated.

Histopathological Evaluation of Mucosa

Histopathological evaluation of tissue incubated in PBS (pH 6.6) after collection was compared with tissue incubated in the diffusion chamber with *in situ* gel formulation. Tissue was fixed in 10 % buffered formalin (pH 6.6), routinely processed and embedded in paraffin. Paraffin sections (7 µm) were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope, to detect any damage to the tissue during *in vitro* permeation by a pathologist blinded to the study.²⁵

In vitro Antibacterial Activity

Formulation G6 containing satranidazole as drug are used in microbial assays. Drug equivalent to 1 mg formulations are used for measurement of zone of inhibition. Under aseptic conditions the formulated gels and placebo were placed on blood agar plates containing porphyromonas gingivalis and were incubated at 37°C for 24 h, after which zone of inhibition was measured. This was continued for 3 days and zone of inhibition on every 24 h interval was measured.²⁶⁻²⁷

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of the *in situ* gel was performed to evaluate the surface and cross morphology of the *in situ* gel (Joel jsm-6490la analytical SE). Samples were dried for 24 h before the analysis. Samples were transferred into the SEM instrument after gold coating. The SEM analysis was carried out at room temperature.

Stability Studies

The optimized *in situ* gel formulation F5 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.

RESULTS

In this study, the thermo gelling polymer, chitosan with different concentrations has been used for formulation of *in situ* gel of Satranidazole with 10 % propylene glycol as plasticizer and were allowed to cross linked with 1 % solution of glutaraldehyde in order to extend the drug release for a longer period of time. The typical composition is shown in Table 1. The solubility studies of pure Satranidazole as shown in Table 2 revealed that the pure drug is freely soluble in methanol, dilute acid, 1,4-dioxane and n, n- dimethyl formamide and practically insoluble in water. The FTIR spectra of Satranidazole-chitosan physical mixture (Figure 1) shows characteristic peaks at 3000 (aromatic –CH stretch), 1750 (C=O stretch), 1350(-NO₂ Stretching) and 750 cm⁻¹ (NO₂ Stretch) respectively. All characteristic peaks of drug are present in the polymer drug mixture. There is reduction in peak intensity but not shifting of the band indicating that there is no interaction between satranidazole and the respective polymer, hence compatible. X-ray diffraction was carried out to determine the nature of the materials whether a material is amorphous or crystalline. Pure Satranidazole showed characteristic peaks at 16.764 °, 66.44 °, 74.25 °, 51.50 ° at 2θ value. The XRD of drug – chitosan physical mixture (Figure 2) revealed a reduction in polymer peak intensity when compared with XRD of plain drug and polymer and showed reduced crystalline properties, indicating of possible conversion into amorphous form. A little decrease in the crystallinity of drug polymer physical mixture is due to the hydrogen bonding between the drug and polymers, which leads to their good compatibility. The developed formulations were evaluated for various parameters like surface pH, gelation temperature, drug content, spreadability, viscosity, bio adhesion study, *in vitro* drug release and *in vitro* antibacterial activity. The formulation stored at 4°C before application, which is syringeable through 21 gauge needle. This formulation is directly injected in to periodontal pocket where it will immediately convert in to gel form at body temperature. In the preliminary studies, the minimum concentration of chitosan that formed gel below 36°C was found to be 0.5 % wt/vol. In general, the gelation temperatures have been considered to be suitable if they are in the range of 25°C to 37°C. If the gelation temperature of a thermo gelling formulation is lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37°C, a liquid dosage form still exists at the body temperature, resulting in the loss of the administered drugs at an early stage. As the temperature of the periodontal cavity is 36°C, this study aimed at preparing the liquid formulations of chitosan that may gel below 36°C. The results also revealed that as the increase of bio adhesive polymer chitosan concentration, increase in the gelation temperature. The pH of all the formulations were found to be within the range between 5.8 – 6.5 and the periodontal mucosa can tolerate the above mentioned pH of the formulations.

Table 1: Composition of different cross linked *in situ* gel formulations containing satranidazole

Ingredients	G1	G2	G3	G4	G5	G6
Satranidazole	1 %	1 %	1 %	1 %	1 %	1 %
Chitosan	0.5 %	1 %	1.5 %	2 %	2.5 %	3 %
Propylene glycol	10 %	10 %	10 %	10 %	10 %	10 %
Acetic acid solution (1%)	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Aq. Gluteraldehyde	1 %	1 %	1 %	1 %	1 %	1 %

Table 2: Solubility Profile of Pure Satranidazole Drug in various solvents

S. No.	Solvent	Solubility (mg/ml)
1	Water	0.01
2	0.1N HCl	0.65
3	6.6 PH	0.55
4	7.4 PH	0.57
5	Sorbital	0.20
6	Glycerol	0.46
7	PG	1.14
8	Tween 80	5.85
9	PEG 400	11.42

Table 3: Summary of evaluated parameters for various Satranidazole *in situ* cross linked Gel

Formulation code	Surface pH	Spreadability (g cm/sec)	Viscosity (dynes/ cm ²)	Gelation temp (°c)	Drug content (%)
G1	5.6 ± 0.57	25.15 ± 1.24	1380 ± 0.25	35 ± 0.89	87.5 ± 0.85
G2	5.8 ± 0.45	25.15 ± 1.53	1435 ± 0.84	34 ± 0.65	86 ± 0.55
G3	5.9 ± 0.05	25.15 ± 1.12	1515 ± 0.52	35 ± 0.43	93.8 ± 0.32
G4	5.86 ± 0.17	25.15 ± 1.85	1714 ± 0.31	35 ± 0.29	95.2 ± 0.74
G5	6.15 ± 0.24	25.15 ± 0.67	1790 ± 0.22	36 ± 0.10	96.1 ± 0.65
G6	6.26 ± 0.05	25.15 ± 0.24	1815 ± 0.14	36 ± 0.05	98.6 ± 0.42

Each value indicates the mean ± SD (n = 3)

Table 4: *In vitro* release Kinetic Model-Fitting Profile of Optimised G6 Formulation

Formulation code	Kinetic models								Best Fit Model
	Zero order		First order		HIGUCHI		PEPPAS		
	R	K ₀	R	K ₁	R	K _H	R	n	
G6	0.985	0.696	0.893	-0.011	0.829	-51.8	0.962	1.05	Zero order

Table 5: Determination of MIC of the various formulations

Formulations	Minimum Inhibitory Concentration (µg/ml)
Satranidazole standard	0.5
Blank <i>in situ</i> gel formulation	-
<i>In situ</i> cross linked gel formulation	0.5

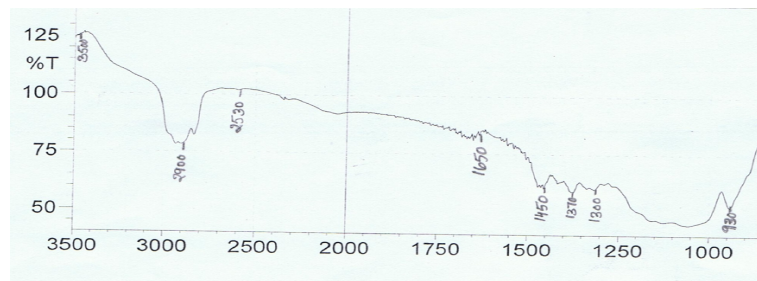


Figure 1: FTIR of Drug Chitosan Mixture

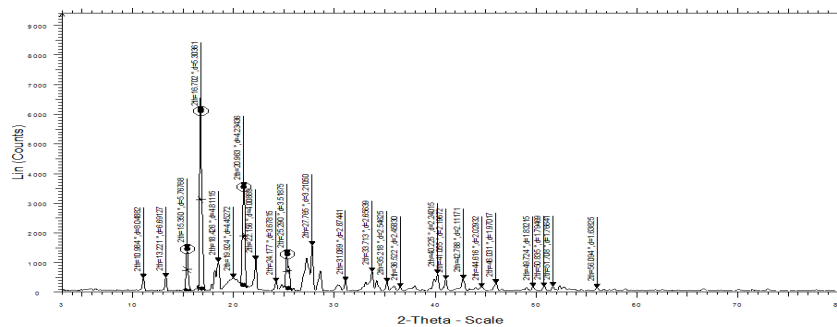


Figure 2: XRD of Drug - Chitosan Mixture

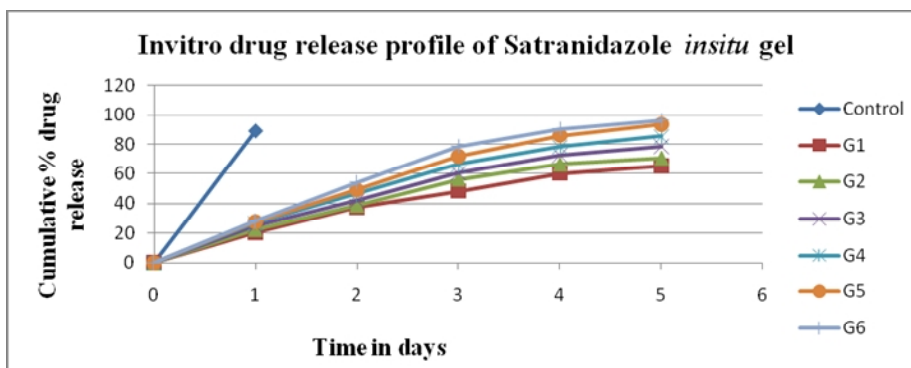


Figure 3: *In vitro* release profile of *In situ* cross linked periodontal gel formulations with control

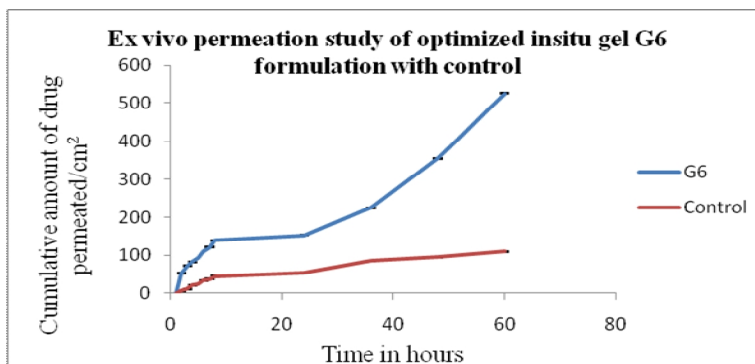


Figure 4: Ex -vivo permeation profile of optimized *insitu* gel G6 formulation

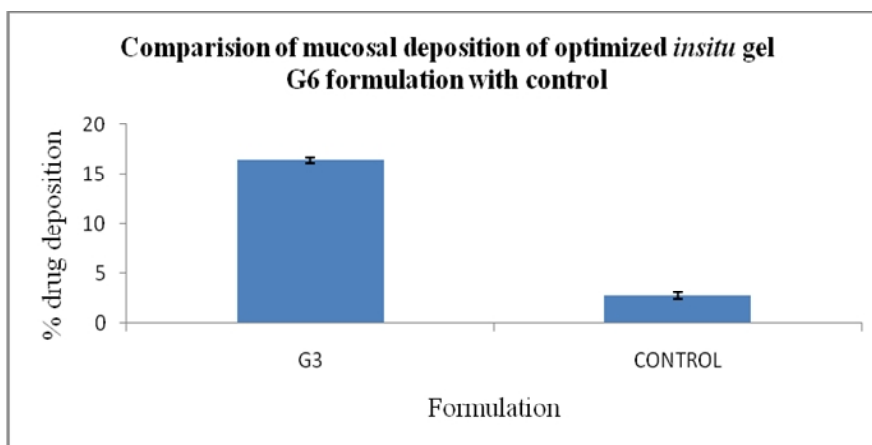


Figure 5: Comparison of mucosal deposition of G6 formulation with control

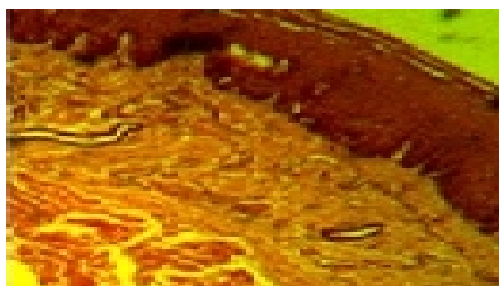


Figure 6: Photographs of histology of mucosa treated with optimized *insitu* gel G6

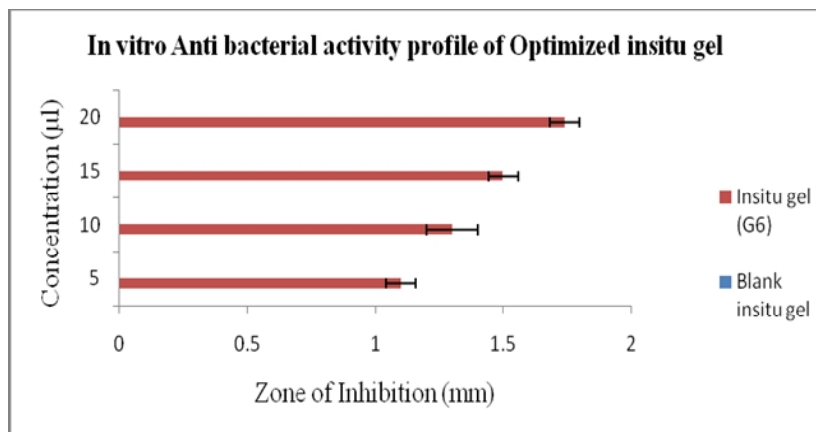


Figure 7: *In vitro* Anti bacterial activity profile

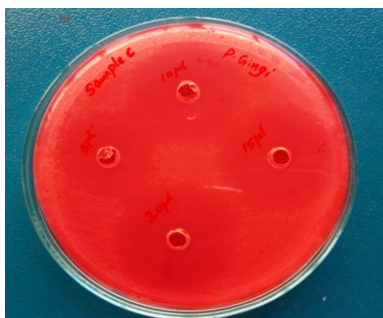


Figure 8: Photo graph of *In vitro* Antibacterial Activity of *In situ* gel (G6) formulation

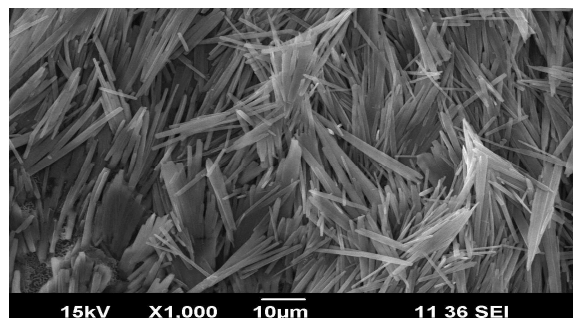


Figure 9: Scanning electron microscopy of cross linked *in situ* G6 gel formulation

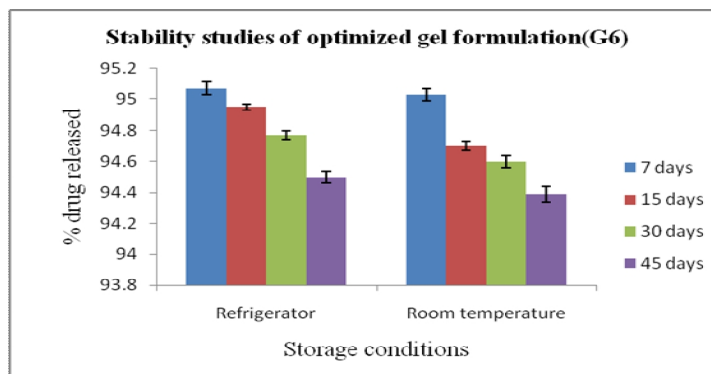


Figure 10: Stability Study of cross linked *In situ* gel formulation (G6)

The formulated gel shows good spreadability indicates uniform spreading of drug when applied. Viscosity of prepared gel was found to be significantly affected by cross linking on positive side, increase in concentration or increase in molecular weight of chitosan beyond this, resulted in drastic increase in viscosity. As the concentration of polymer increases, viscosity increases proportionally. All these chitosan formulations offer good syringe ability and optimum viscosity characteristics. At gelation temperature, liquid phase makes transition into gel. Drug content uniformity in the drug delivery system is an important aspect that determines the performance of the system *in vivo* conditions. If the drug is not distributed uniformly throughout the formulation, it could either lead to availability of sub therapeutic dose or toxic dose. The formulation exhibited fairly uniform drug content was found to be 86 % - 98.6 %.

The results obtained *in vitro* release studies were plotted in Cumulative percent drug release Vs. Time with greatest release for G6 formulations (Zero order rate kinetics) shown in Figure 3. *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 5 days for different formulations, sufficient to inhibit the growth of the micro-organisms. Considering the initial pathological load in the periodontal pocket, an initial burst release of anti microbial is always desirable. After five days the gel had lost its gelling property and hence was not fit for further release study. The maximum cumulative percentage amount of drug release value for control was only $55.58 \pm 1.087\%$ in 24th hour. *In vitro* drug release kinetic analysis (Table 4) showed that release mechanism for the *in*

in situ gel G6 fitted best to the zero order model, as the plots showed high linearity ($R^2 = 0.985$). Drug permeation studies through bovine periodontal mucosa conducted on the PBS 6.6 solution of the pure drug showed nearly 33 % permeation in less than 48 h for the optimized G6 formulation. Ex vivo permeation studies of Satranidazole from *in situ* gel formulations from Figure 4 indicates slow and sustained permeation of the drug for 1 to 48 h compared to control. Percentage drug deposition in the periodontal mucosa from G6 formulation showed that higher concentration of drug get deposited in the mucosa than control. The comparison of mucosal deposition is shown in Figure 5. The microscopic observations indicate that the optimized G6 *in situ* gel has no significant effect on the microscopic structure of mucosa. As shown in Figure 6, neither cell necrosis nor removal of the epithelium from the periodontal mucosa was observed after permeation of *in situ* gel (G6). The epithelium layer was intact and there were no alterations in basal membrane and superficial part of sub mucosa as compared with PBS-treated mucosa. Thus, the gel formulation seems to be safe with respect to local sub gingival (periodontal) administration. The antibacterial activity of G6 optimized *in situ* gel formulation against *Porphyromonas gingivalis* was compared with blank *in situ* gel formulation. The antibacterial activity was carried out at five different concentrations (2 μ l, 5 μ l, 10 μ l, 15 μ l, 20 μ l) were represented graphically in Figure 7. The photographs were shown in Figure 8. The minimum inhibitory concentrations of the samples were determined and the value was tabulated in the Table 5. Scanning electron microscopy (SEM) of G6 formulation was done to study the morphological characters shown in Figure 9. The stability studies of the optimized cross linked gel formulation (G6) at refrigerator temperature ($4 \pm 2^\circ\text{C}$), room temperature ($30 \pm 2^\circ\text{C}$) and oven temperature ($45 \pm 2^\circ\text{C}$) were carried out for 45 days and were observed for any physical changes, such as colour, appearance, flexibility, or texture, drug content and % drug release was estimated at an interval of one week. The physical appearance showed that it does not show any changes when compared to the freshly prepared formulations at refrigerator and room temperature. The % drug release evaluated for the three formulations on 7th, 15th, 30th, 45th day is represented in Figure 10 showed that there are no significant changes in the drug content uniformity during the storage for 45 days in refrigerator conditions.

DISCUSSION

The present study was aimed to develop a low-dose local controlled delivery system of antibacterial drug Satranidazole in the form of an *in situ* gel to overcome the problems like low solubility, low bioavailability and half-life of the drug, thereby prolonging the duration of action for treating periodontal infections and to maintain the concentration of the drug above its minimum inhibitory concentration for a prolonged period of time at the site of infection. When the polymer solution was exposed to gingival crevicular fluid, solvent diffused away from the polymer-drug mixture and fluid diffused into the mixture where it coagulated the polymer thereby trapping or encapsulating the drug within the polymeric matrix. The result revealed that propylene glycol and glutaraldehyde in formulation could testify the *in situ* forming effect. The FTIR studies and the XRD studies confirmed the absence of chemical interaction between the drug and the polymer. The various physio-chemical data confirmed that *in situ* gel drug delivery system can be developed by using natural polymer chitosan. Thermo gelling

polymer-based liquid formulations that provide *in situ* gelling property in periodontal cavity were designed to delay clearance of the formulations from the oral cavity. Best formulation in terms of cumulative percent drug release along with zero order kinetic mechanism was formulation G6 with 98.6 % drug release for 5 days and fulfilled many requirements of once a week delivery system, easy to fabricate, cost effective patient compliance is also very high. The *in vitro* release studies concluded that the drug(s) were found to release at a constant rate in a controlled manner with reproducible kinetics, therefore chitosan may be a useful matrix for sustained release of drugs, and cross-linking of the polymer is essential for the management of periodontitis. Throughout the permeation study, the average permeation rate for *in situ* gel was found to be above the minimum inhibitory concentration of Satranidazole indicating the suitability of formulating Satranidazole as a controlled release local delivery *in situ* gel for longer periods of time. The microscopic observations indicate that the *in situ* gel has no significant effect on the microscopic structure of mucosa. Histopathological study of the periodontal mucosa after permeation study suggested that the gel formulations were safe for local anti microbial treatment in to the infected periodontal pocket. The *in vitro* antibacterial activity demonstrated a significant antibacterial profile of the *in situ* gel G6 formulation against *Porphyromonas gingivalis*. The activity is due to enhanced penetration of cross linked chip containing drug through the bacterial cell wall produces extensive DNA damage characterized by helix destabilization and strand breakage. The SEM of *in situ* gel suggesting that the drug(s) were dispersed rather than dissolved in the polymer matrix. From the stability studies it was confirmed that the *in situ* gel formulation of Satranidazole remained stable at room temperature ($30 \pm 2^\circ\text{C}$) and refrigerator temperature ($4 \pm 2^\circ\text{C}$) when compared with elevated temperature. In addition, all these findings suggested that the cross linked optimized *in situ* gel formulation (G6) was proved a suitable alternative for the local antimicrobial delivery into the infected periodontal pocket.

CONCLUSION

The study proved that gel implants as *in situ* drug system can be developed by using natural thermo gelling polymer, chitosan for periodontitis because of its sustain release profile, water-soluble nature, physical stability and good spreadibility. The gel formed *in situ* afforded sustained drug release over 5 days period. The local drug delivery of satranidazole may be beneficial since it would probably eliminate side effects, also adheres with gums for a prolonged period of time, reduces the dosing frequency, and lowers the bitterness of the periodontal gel, which occurs with systemic dosing. Furthermore, studies are essential to evaluate the clinical efficacy, patient acceptability, and compatibility of the designed chips for the effective treatment of periodontitis.

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