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DEVELOPMENT AND EVALUATION OF PERIDONTAL STRIPS OF ORNIDAZOLE

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Abstract:
The aim of the present study was to develop site specific drug delivery of ornidazole for the treatment of periodontitis which has excellent activity against anaerobic microorganisms. The formulations were prepared by utilizing polymers such as PEO and gellan gum by solvent technique with the used of plasticizer (PEG-400). The calibration curve of ornidazole was developed in PBS pH 6.8 at 317nm in the range of 2 to 20 µg/ml. Compatibility study were carried out by FT-IR and Differential scanning Colorimetry. The formulations were evaluated for thickness, folding endurance, weight variation, drug content, percent moisture loss, tensile strength, and percent elongation, SEM and in vitro antibacterial activity. In vitro drug release study was also carried out using PBS pH 6.8 and the samples were analyzed by UV-spectrophotometrically at 317nm. FT-IR and DSC study revealed no interaction between drug and polymers. Formulations shown good uniformity of drug content, there is no any kind of effect on moisture loss test. Formulations showed thickness within the range of 0.0417 to 0.0633. Formulations F3, F4, F7 and F8 showed good tensile strength. By increasing the concentration of PEO in the formulation increases the tensile strength, percent elongation and folding endurance. Formulation F3 released 92.44% of drug at the end of three minute and was considered as best formulation. A short-term stability study of the optimized formulation F3 was carried out at 40^0C for three months. At periodic interval sample were analyzed for drug content and in vitro drug release study, result showed 91.66% release at the end of 90days.
Keywords: *Ornidazole, dental strip, Physical characterization, in vitro release, antibacterial study.*

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INTRODUCTION:

Periodontitis, also known as gum disease, is a set of inflammatory disease affecting the tissues surrounding the teeth. Periodontitis involves progressive loss of the alveolar bone around the teeth, and if left untreated, can lead to the loosening and subsequent loss of teeth.

Periodontitis is caused by microorganism that adheres to and grow on the tooth's surfaces, along with an over-aggressive immune response against this microorganism. A diagnosis of periodontitis is established by inspecting the soft gum tissues around the teeth with a probe and by evaluating the patient's X-ray films, to determine the amount of bone loss around the teeth [1].

Conventional therapy, surgery, based on scaling, surgery and the administration has been proposed [2]. However, the systemic route of antibiotic administration may not be ideal because of the concern over the development of bacterial resistance that may be induced over longer period of time. Systemic antibiotic therapy over a long period of time also raises the risk of undesirable side effects such as nausea, fever, abdominal pain and pseudomembranous colitis [3].

But local delivery of antimicrobial agents is becoming more prevalent since it leads to higher concentration of the drug at the intended site of action using a lower dose with an associated reduction in side effects related to systemic administration [4].

Ornidazole is an antibiotic used to treat some protozoan infections. It has also been investigated for use in Crohn's disease after bowel resection [5]. After passive absorption into bacterium cell, the nitro group of ornidazole is reduced to an amine group by ferrodxin-type redox systems. The formation of redox intermediate intracellular metabolites is believed to be the key component responsible for killing microorganisms. The drug is active against anaerobic bacteria including *Peptostreptococcus, Clostridium, Bacteroides fragilis, Prevotella, Porphyromonas gingivalis*, and *Fusobacterium* as well as protozoa including *Entamoeba histolytica, Trichomonas vaginalis* and *Giardia lamblia [6]*.

The present study was aimed to formulate site-specific controlled release dental strips for the treatment of periodontal diseases. The various pharmaceutical parameters like thickness, compatibility studies, tensile strength, uniformity, weight, content uniformity, percentage elongation, folding endurance, DSC, percent moisture loss and SEM. In vitro antibacterial activity and stability studies were performed on optimized formulation.

MATERIALS AND METHODS:

Ornidazole was obtained as a gift sample from Zydus Cadila Health Care Ltd, Ahmedabad, India. Polyethylene glycol 400, Calcium chloride, Sodium hydroxide and Sodium dihydrogen phosphate were obtained from Loba chemicals, Mumbai, India. Polyethylene oxide was obtained from Aldrich chemistry, Gellan gum was obtained from Sigma Life Science and Agar was obtained from Merk chemicals, Mumbai India.

PREPARATION OF ORNIDAZOLE STRIPS:

The formulations of Ornidazole were prepared in the laboratory using the polymer such as polyethylene oxide and gellan gum with the use of plasticizer (PEG-400) by solvent casting method. Ornidazole was dissolved in 10ml distilled water containing polyethylene oxide which is previously dissolved by putting the solution on magnetic stirrer (rpm 30/min). In another side the gellan gum is dissolved in a 5ml distilled water by warming. Then both the solutions were mixed and plasticizer PEG 400 was added, stirred well on magnetic stirrer (rpm 30/min). To the above solution was allowed to stand for 30min for deaeration. The solution was casted on a petridish (diameter 8.8cm) and dried at room temperature for 24hr. The film was carefully removed from the petridish, checked for any imperfections and cut into the required size to deliver the equivalent dose (7X7mm^2 per strip) containing of 1mg of drug. The samples were stored in a desiccator at relative humidity 30-35% until further analysis.

Table 1: Composition of Different Formulations Containing Ornidazole

Sr. No	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
1	Ornidazole (mg)	159	159	159	159	159	159	159	159
2	PEO (mg)	150	175	200	225	150	175	200	225
3	Gellan gum (mg)	100	75	50	25	100	75	50	25
4	PEG-400 (ml)	5%	5%	5%	5%	10%	10%	10%	10%
5	Water (ml)	15	15	15	15	15	15	15	15

EVALUATION OF ORNIDAZOLE STRIP FORMULATION

Thickness Content Uniformity:

The thickness of strips was measured by using Digimatic Micrometer (Mitutoyo, ABSOLUTE). The thickness of each strip was determined at six different places and the average was calculated. The standard deviations of thickness were computed from the mean value [7].

Drug Content Uniformity:

To check the uniformity of the drug in the strip (7 X 7 mm^2), three strips were taken out from each batch. Each strip was then placed in volumetric flask containing 10ml of distilled water and shaken to extract the drug from strip. One millilitre of above resulting solution was withdrawn, after suitable dilution with distilled water and analyzed UV-spectrophotometrically at 317 nm using distilled water as blank. The mean and standard deviation of drug content of three randomly selected strips were calculated. The same procedure was adopted for all the batches and drug content was noted [9, 10].

Weight Uniformity:

Strips (size of 7X7 mm^2) were cut from different areas of film. The weight of each strip was taken and weight variation of six strips was calculated. The standard deviations of weight were computed from the mean value [8].

Folding Endurance:

One strip was folded up to 300 times or folded repeatedly at the same place till it broke, which is considered satisfactory to reveal good strip properties by this method folding endurance was determined. The value of the folding endurance is the no. of times strip folded at a place without breaking. This test was done on all the batches five times [2].

Percentage Moisture Loss Test:

Percentage Moisture loss was determined by keeping the strips (7X7 mm^2) in a desiccator containing anhydrous calcium chloride. After 3 days, the strips were taken out, re-weighed and the percentage moisture loss was calculated using the following formula.

Percentage Moisture Loss = Initial weight- Final weight/Initial weight X 100

Tensile Strength and Percentage Elongation:

Tensile strength and percentage elongation of the strips was determined with 'Texture analyzer' testing machine. It consists of two load cell grips. The lower one is fixed and upper one is movable. The test strip of specific size (4 X 1 cm^2) was fixed between these cell grips and force was gradually applied till the strip breaks. The tensile strength of the strips was taken directly from the dial reading. The percentage elongation of strips was calculated by applying the following equation. Same procedure was repeated for three times and standard deviation was calculated form mean values.

Tensile Strength = Load at failure X 100/ Strip thickness X Strip width

Percentage elongation = Increase in length/Original length X 100

Surface Electron Microscopy:

The morphology of strips were determined using SEM (JSM 5610 LV, Jeol Datum Ltd. Japan) operated at an accelerating voltage of 10kV.

***In vitro* drug release study:**

In vitro drug release study was performed by placing strips of known weight and dimension (7 X 7 mm^2) into small beaker containing 10ml of PBS pH 6.8. The beaker was placed on magnetic stirrer at 30rpm and temperature was set to 37˚C. At periodic interval the samples were taken and the drug content was analyzed at 317 nm against reference standard using PBS pH 6.8 as a blank on a UV-Visible spectrophotometer (Shimadzu Inc., Japan). Then immediately known amount of PBS pH 6.8 was added. The same procedure was repeated for three times. In vitro release data obtained was plotted and tabulated [11].

***In Vitro* antibacterial activity:**

In vitro antibacterial activity was performed on optimized formulations by placing the strip (0.5 X 0.5 cm) on agar plates seeded with the oral bacteria '*Streptococcus aureus'* and E. Coli. After 48h of incubation at 37˚C, the films were transferred to freshly seeded agar plates and incubated for an additional 48h. This procedure was repeated until no inhibition of bacterial growth was detected on the agar plate. The growth inhibition zone on the agar plate was measured [13].

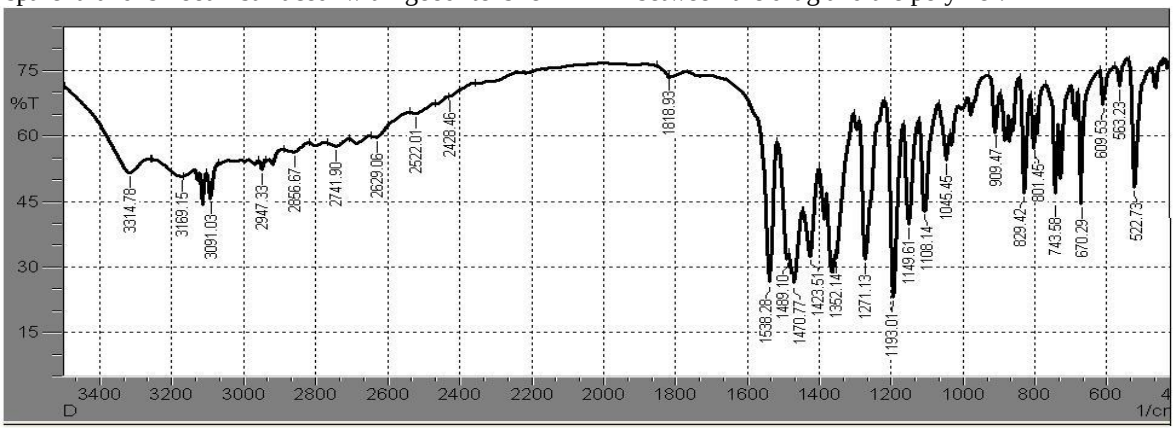
Stability study:

Stability studies were carried out on formulation F3, according to ICH guidelines by strong replicates of strips (packaged in aluminium foil) in a humidity chamber, with a releative humidity of 75±5% and a temperature of 40±0.5˚C. At periodic intervals the samples were taken out at 0, 15, 45 and 90 days and the period for their degradation of the strip was checked. Samples were also analyzed for drug content [12].

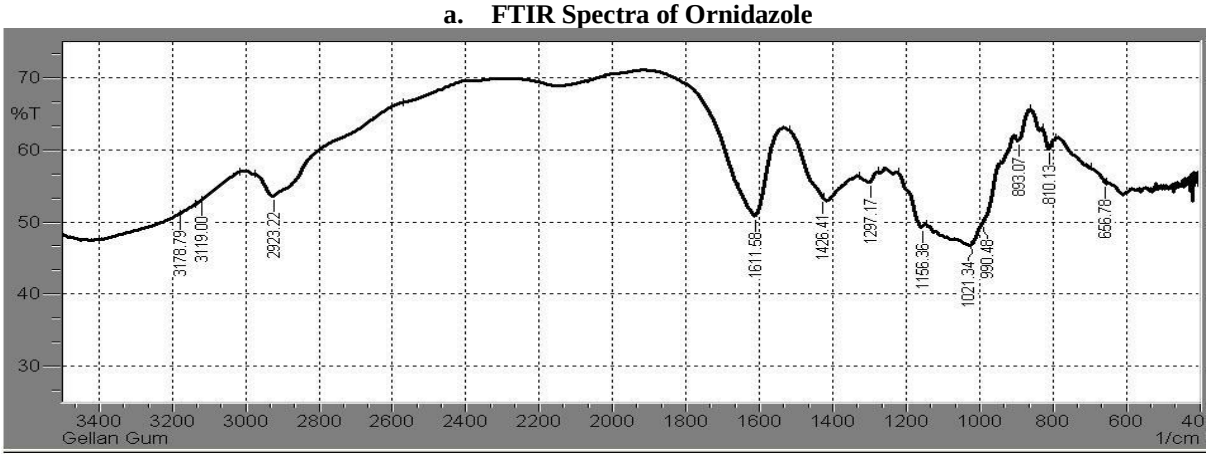
RESULT AND DISCUSSION:

In the present study, periodontal strips of Ornidazole were formulated using polymers such as PEO and gellan gum by solvent technique with the used of plasticizer (PEG-400). The prepared strips were transparent and smooth surfaced with good tensile strength. The procedure developed to prepare the strips was reproducible.

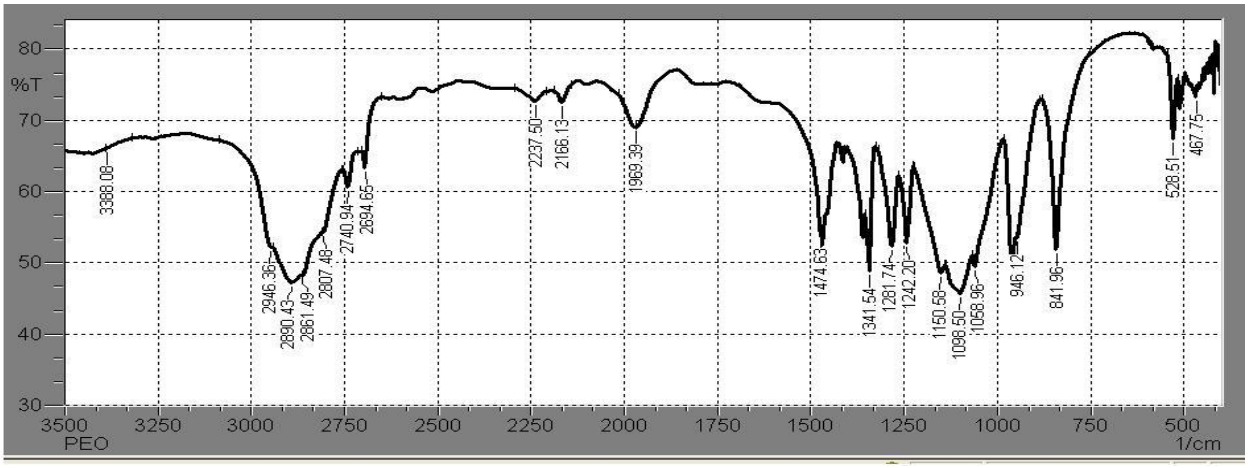
The results of FTIR studies are shown in Figure 1, confirmed the absence of any chemical interaction between the drug and the polymer.



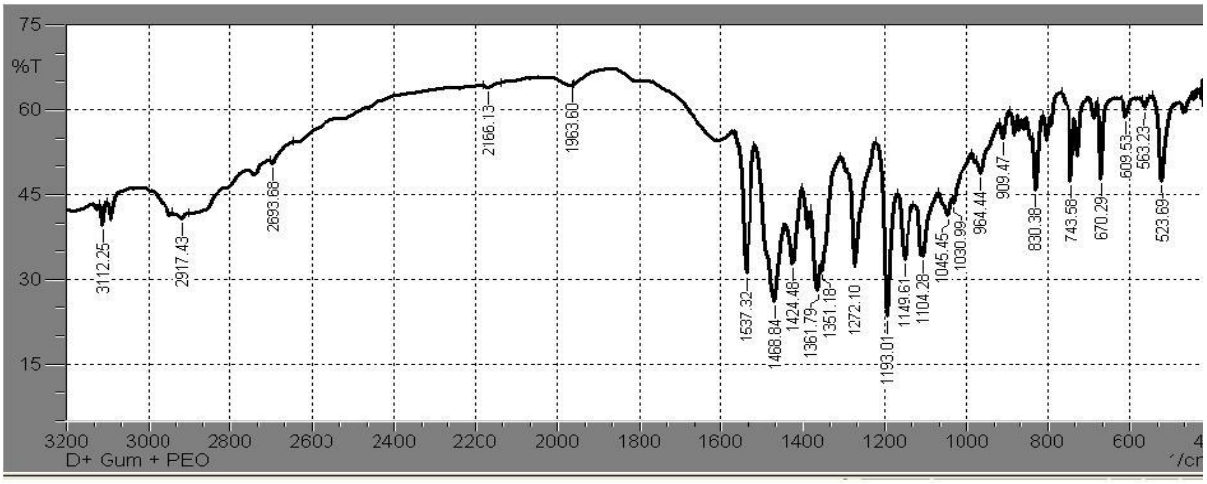
a. FTIR Spectra of Ornidazole



b. FTIR Spectra of Polyethylene Oxide



c. FTIR Spectra of Gellan Gum



d. FTIR Spectra of Ornidazole And Excipients

Fig. 1: FTIR Spectra of a. FTIR Spectra of Ornidazole b. FTIR Spectra of Polyethylene Oxide c. FTIR Spectra of Gellan Gum d. FTIR Spectra of Ornidazole And Excipients

Thickness Uniformity:

The thicknesses of drug-loaded strips were measured with the help of Digimatic Caliper. The mean values were shown in the Table 2.

Table 2: Thickness Determination Of Strips

Sr. No.	Formulation code	Average thickness (mm) AM±SD
1	F1	0.0567±0.0052
2	F2	0.0533±0.0052
3	F3	0.05±0.0058
4	F4	0.0417±0.0041
5	F5	0.0633±0.0052
6	F6	0.0617±0.0041
7	F7	0.0567±0.0052
8	F8	0.0417±0.0041

*Each reading is an average of six determinations

There were no significant changes in the thickness of strips loaded with polyethylene oxide and gellan gum. The concentration of plasticizer did not alter the change in the thickness of strips.

Weight Uniformity:

Drug loaded strips (7 X 7 mm^2) were tested for uniformity of weight and the results are given in the Table 3. The weight of all the prepared batches was found to quite uniform. Standard deviation of all the strips ranged between 0.06324 and 0.13784. The change in the concentration of polymers and plasticizer did not show the difference in the weight of strips.

Table 3: Weight Uniformity of Ornidazole Strips:

Sr. No.	Formulation code	Average weight (mg) AM±SD
1	F1	3.05±0.104881
2	F2	3.1±0.089443
3	F3	3.15±0.083666
4	F4	3.03±0.121106
5	F5	3.05±0.13784
6	F6	3.3±0.063246
7	F7	3.016±0.09831
8	F8	2.9±0.063246

*****Each reading was an average of six determinations.

Folding endurance:

Strips did not show any cracks even after folding for more than 250 times. The change in the concentration of polymers and plasticizer did not show the difference in the folding endurance of strips. Hence it was taken as the end point.

Percentage moisture loss test:

This test is of great significant in moisture content causes a significant variation in mechanical properties of the strip especially, when the strip comprises of hydroscopic components. The capacity of the strip to take up water is an intrinsic parameter of the polymeric system in consideration to the release of drug.

Table 4: Percentage Moisture Loss of Ornidazole Strips

Sr. No.	Formulation code	Average weight (mg) AM±SD
1	F1	7.42223 ± 1.705505
2	F2	5.592667 ± 3.81731
3	F3	5.4459 ± 1.930701
4	F4	2.298667 ± 1.990704
5	F5	5.235335 ± 1.9132
6	F6	6.5948 ± 0.12453
7	F7	4.558167 ± 2.025919
8	F8	4.679767 ± 1.919747

*Each reading was an average of the determinations.

Drug Content Uniformity:

The content uniformity test is commonly employed for unit dosage forms. In order to make sure about the uniform dispersion of drug in strips, the drug content was carried out. The drug content was analyzed at 317 nm by using suitable blank. The drug is not lost as indicated by more than 80% of drug loading in all the formulations. In Table 5 AM ± SD were expressed in the results. The drug was uniformly dispersed as shown in the result.

Table 5: Drug Content Uniformity of Ornidazole Strips

Sr. No.	Formulation code	Amount of drug present (mg)	%Drug present
1	F1	135.67	85.3333±0.030551
2	F2	140.97	88.6667±0.015275
3	F3	142.56	89.6667±0.025166
4	F4	129.84	81.6667±0.051316
5	F5	127.40	80±0.02
6	F6	130.38	82±0.036056
7	F7	135.15	85±0.043589
8	F8	134.62	84.667±0.015275

*****Each reading is an average of three determinations.

Tensile Strength test:

Tensile strength was determined using texture analyzer for the drug loaded strips. The data for tensile strength are given in the Table 6. The tensile strengths of drug loaded strips are in the order of F2 > F1 > F3 > F4 > F5 > F6 > F7 > F8.

Table 6: Tensile Strength of Ornidazole strips

Sr. No.	Formulation code	Tensile strength (kg) AM±SD
1	F1	1.44±0.01
2	F2	1.27±0.03
3	F3	1.87±0.01
4	F4	1.92± 0.03
5	F5	1.93±0.03
6	F6	2.13±0.01
7	F7	2.48±0.01
8	F8	2.71±0.03

The concentration of polymer and plasticizer shows higher effect on tensile strength of strip. When the concentration of polyethylene oxide increases accordingly then the tensile strength also increases. As per literature survey the plasticizer shows effect on tensile strength, in our study when the concentration of plasticizer increases the tensile strength also increases.

Percentage elongation test:

Percentage elongation was determined using universal texture analyzer for the drug loaded strips, as shown in Table 7. The order of percentage elongation of the strips is F1 > F2 > F5 > F3 > F4 > F6 > F7 > F8.

Table 7: Percentage elongation of Ornidazole strips:

Sr. No.	Formulation code	Percentage elongation (%)
1	F1	17.13±1.52
2	F2	19.57±1.85
3	F3	24.73±1.85
4	F4	28.22±1.98
5	F5	23.22±1.98
6	F6	31.02±1.71
7	F7	32.89±0.85
8	F8	36.04±1.74

Scanning electron microscopy of optimized formulations:

Scanning electron microscopic images of optimized formulations were shown in Figure 2, 3 and 4.

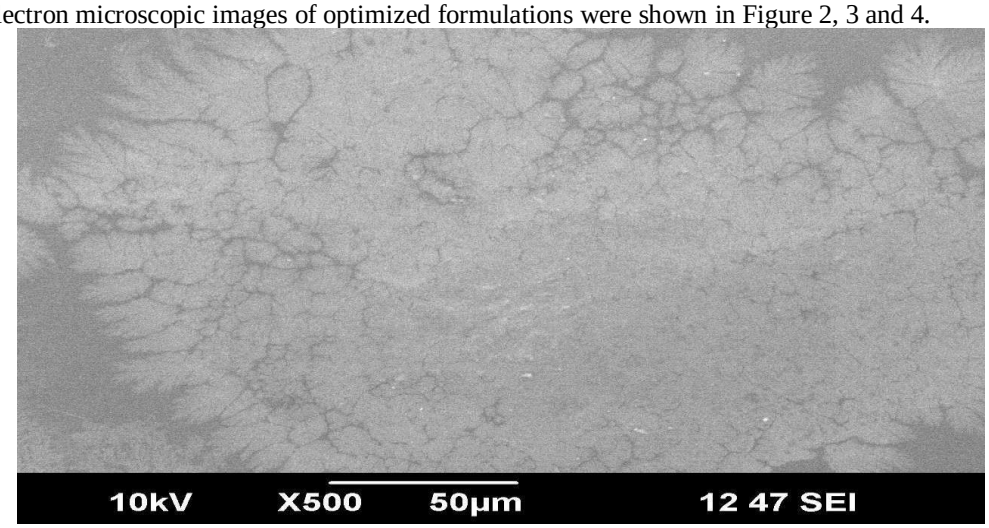


Fig. 2: SEM images of 50 um

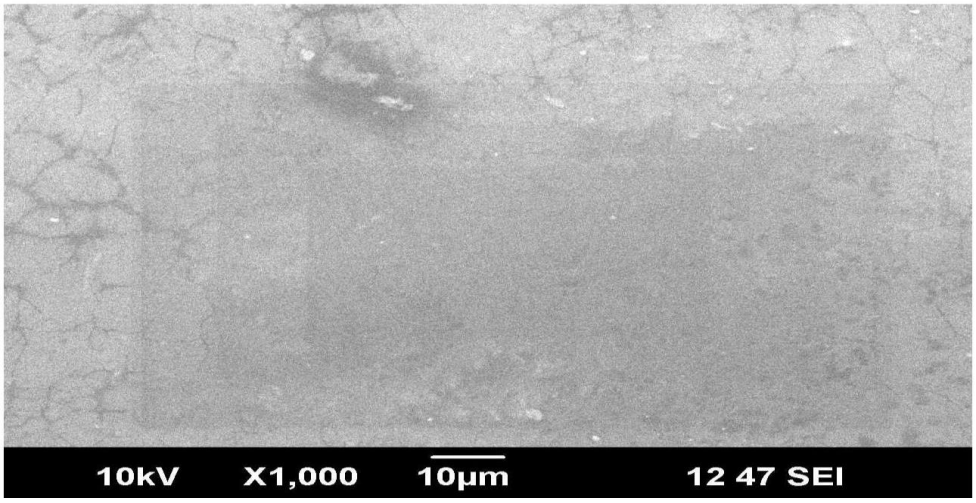


Fig. 3: SEM images of 10 um

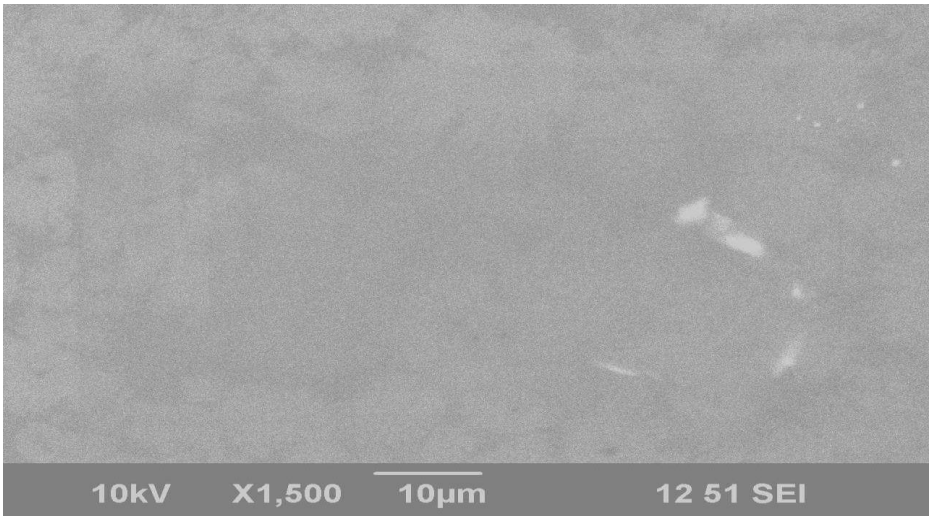


Fig.4: SEM images of 10 um

The SEM study of optimized batch shows the smooth surface of strip and there was uniform distribution of drug throughout the formulations.

In-vitro drug release study:

In vitro drug release study of various formulations was carried out in PBS pH 6.8; the release data of formulations were shown in Table 8.

Table 8: In vitro drug release study:

Time (sec)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
30	-	-	28.217	28.479	-	-	-	37.130
60	19.25	24.14	46.731	47.278	24.831	22.435	29.897	48.757
90	-	-	62.308	62.149	-	-	-	59.328
120	29.545	33.186	75.970	80.948	34.231	40.355	46.807	75.713
180	38.502	47.262	92.441	88.804	43.630	48.805	63.041	91.041
240	55.748	65.986	-	-	64.393	68.181	76.298	-
300	74.732	81.740	-	-	77.441	84.353	89.556	-
360	88.368	91.813	-	-	87.962	93.677	-	-

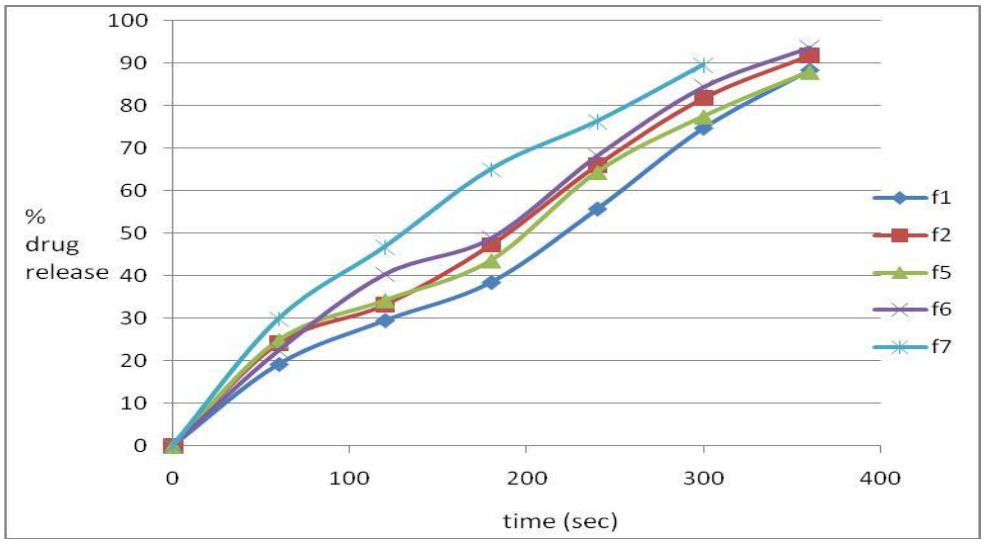


Fig. 5: *In vitro* drug release study of formulation F1-F2, F5-F7

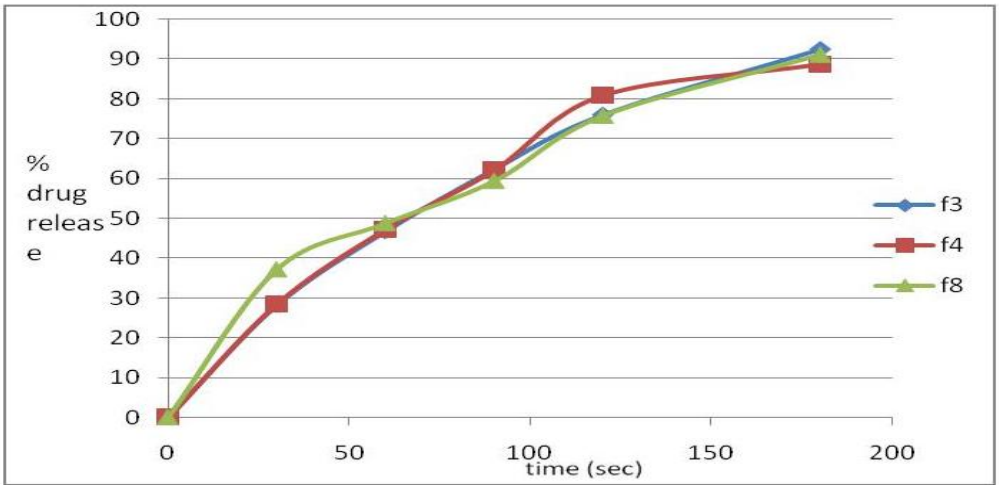


Fig. 6: *In vitro* drug release study of formulation F3-F4, & F8.

In vitro drug release study shown in Figure 5 and 6 indicated that the release of drug varied from the formulation batches according to the type and concentration of polymers utilized. The release of drug was decreased as increase in gellan gum concentration. The drug release was increased due to the concentration of polyethylene oxide shows effect in increases amount of release of drug. The variation of plasticizer concentration does not show any drastic effect on release of drug.

The F3 batch shows higher the drug release within 3 minutes. The F3 batch contains 200mg polyethylene oxide and 50mg gellan gum which shows effect in increases amount of release of drug. The 5% of plasticizer concentration shows increase amount of release of drug.

***In-Vitro* antibacterial activity:**

In vitro antibacterial activity was carried out as mentioned in methodology on *S. aureus* and *E. coli* organism. The results were shown in the table 19 for antibacterial activity. Figure 7 shows the zone of inhibition of the optimized strip against *S. aureus* and *E. coli* on 48 hours.

Table 9: Zone of inhibition of optimized batch

Zone of inhibition (mm) in 48hrs	
S. Aureus	E. coli
24	18

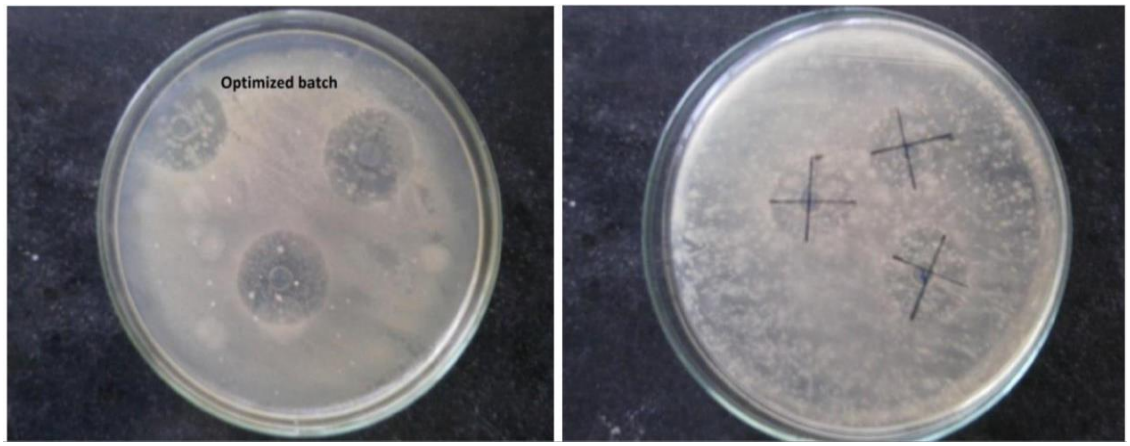


Fig. 7: *In –Vitro* antibacterial activity of *S. AUREUS and E. COLI*

The study indicates that the *S. aureus* and *E. coli* formulated polymeric strips containing ornidazole retained their antibacterial activity.

Table 10: Evaluation parameters of stability batch (F3)

Evaluation parameters	Before stability Storage	After 15 days storage	After 45 days storage	After 90 days Storage
Drug Content (%)	89%	86%	88%	90%
Percent drug dissolve in 6.8pH phosphate buffer	92.441%	91.569%	88.849%	91.667%

There was no significant decrease in drug release and drug content rate of formulation F3 over the period of 3 months.

Stability study of optimized formulation:

Accelerated stability studies were performed at 40 ^{0}C/75% RH as per the ICH guidelines. At periodic interval, the formulation was analyzed for drug content as well as *in vitro* drug release study. Results were showed in Table 10, as no major differences was their between evaluated parameters before and after ageing/storing and all were found to be in acceptable limits. Based on the results of initial characterization batch F3 were thought to be the superior formulation and hence further subjected to accelerated stability study for 3 months.

CONCLUSION:

In conclusion we can say that, the formulated periodontal strip as a drug delivery system promising the approach which is utilized for improving therapeutic efficacy of ornidazole in the treatment of periodontitis. The use of polymer such as polyethylene oxide, gellan gum and plasticizer PEG-400 as well as distilled water as a solvent by using solvent casting method showed better drug release profile at the end of 3 min. Ornidazole was showed a good antibacterial activity. Inclusion of ornidazole also improve therapeutic efficacy of formulation in the management of periodontitis. Hence in future such type of drug delivery system may utilize for the periodontal treatment.

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