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FORMULATION, CHARACTERIZATION AND EVALUATION OF METRONIDAZOLE GEL FOR LOCAL TREATMENT OF PERIODONTITIS

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ABSTRACT

In peridontial disease loss of tooth occures due to weakining of the supporting structure (pocket) of teeth, to prevent this site specific injectable drug delivery systems are required. In the present study six batches of metronidazole gels were prepared using natural biodegradable polymers Chitosan, guar gum and Locust bean gum in variable concentrations. The formulated gels were characterized for surface pH, viscosity, syringeability, bioadhesion strength, in vitro drug release studies and antimicrobial susceptibility test. The results revealed that the surface pH was within the range of neutral pH. The bioadhesion strength was maximum for F3 formulation (3% Chitosan); viscosity values were ranging from 1453.33 ± 5.77 to 1995.00 ± 0.01 dyne/cm^2. Best formulation in terms of cumulative percent drug release along with bioadhesion was formulation F3 with 78.23 % drug release for 7 days and fulfilled many requirements of once a week delivery system, easy to fabricate, cost effective, patient compliance is also very high. Zone of inhibition was also satisfactory for all the formulations.

KEY WORDS

Peridontial disease, Metronidazole, Chitosan, Guar gum, Locust bean gum.

INTRODUCTION

The term “Periodontal Disease” broadly defines several diseases associated with the periodontium. Changes in the microflora, histopathological variations, clinical symptoms and the location of the inflammation help to further delineate periodontal disease. Gingivitis, the moderate stage of the disease, caused by an accumulation of supra gingival plaque is characterized by swelling, light bleeding and redness of marginal gingiva. Periodontitis a more severe stage of periodontal disease, results in the resorption of the alveolar bone and detachment of the periodontal ligaments supporting the tooth. One of the clinical features of the periodontal disease is the formation of a periodontal pocket, which is pathologically deepened sulcus. In normal sulcus, the gap between the gingiva and the tooth is normally between 1 and 3 mm deep. However, during periodontitis, the depth of pocket usually exceeds 5mm [1].

Metronidazole is an important active substance that has been widely used in the treatment of some protozoal and anaerobic bacterial infections. For cases that persist after oral treatment and where resistant trichomonads are suspected, a combination of oral and topical therapy may be effective. Metronidazole is readily

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absorbed from the gastrointestinal tract and metabolized in the liver. This is excreted in the urine, mainly as conjugates and metabolites, and to a lesser extent in the faeces. Bilary excretion may be important in the elimination of Metronidazole and its metabolites. Metronidazole is used in the treatment of hepatic and intestinal amoebiasis, giardiasis, trichomoniasis of urogenital tract and bacterial vaginosis. Also used in the treatment and prophylaxis of susceptible anaerobic infections in dental and gastrointestinal surgery and in other mixed aerobic-anaerobic infections. Metronidazole is also advocated in the management of H.pylori duodenal ulcer in combination with other drugs [2, 3].

Bioadhesive dosage forms have been used to target local disorders at the mucosal surface to reduce the overall dosage required and to minimize the side effects that may be caused by the systemic administration of the drugs. Bioadhesive formulations use polymers as the adhesive component. These polymers are often water soluble and when used in a dry form, they attract water from the mucosal surface and this water transfer leads to a strong interaction. These polymers also form viscous layers when hydrated with water, which increases the retention time over the mucosal surfaces and leads to adhesive interactions [4].

An attempt has been made, in the present work, to develop antibacterial gels of Metronidazole by conventional method, using a bland of natural polymers such as Chitosan, Guar gum and Locust bean gum. The objectives of the study were to investigate the performance of natural polymers and effect on the release characteristics of the Metronidazole (antibacterial) gels.

MATERIALS AND METHODS

Metronidazole was procured from Claris Lifesciences Ltd, Ahmedabad, India. Chitosan sample was obtained from Biological E. Ltd, Hydrabad and Guar gum sample was procured from Loba Chemie Pvt. Ltd., Mumbai. All other Chemicals and solvents were of analytical grade and purchased from local market in India.

***Preparation of Metronidazole gels*[5]:**

Total six formulations were prepared. Placebo gels were prepared by dissolving chitosan at different concentrations in dilute lactic acid (2%) using a mechanical stirrer. Metronidazole was incorporated into the formulations of required concentration by mechanical stirring.

For gel with guar gum, Locust bean gum was completely dissolved in hot water, to which guar gum was added and dissolved using mechanical stirrer to form a gel. Metronidazole was incorporated into the formulations of required concentration by mechanical stirring. Composition of the gel is shown in Table no.1

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Table 1
GEL COMPOSITION

Ingredients	Formulation Code					
	F1 (% w/v)	F2 (% w/v)	F3 (% w/v)	F4 (% w/v)	F5 (% w/v)	F6 (% w/v)
Metronidazole	0.25	0.25	0.25	0.25	0.25	0.25
Chitosan	2.0	2.5	3.0	--	--	--
Guar gum	--	--	--	1.0	1.0	2.0
Locust bean gum	--	--	--	1.0	2.0	1.0
Lactic acid	2.0	2.0	2.0	--	--	--
Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

EVALUATION PARAMETERS OF METRONIDAZOLE GELS

***Surface pH of the gel*[6]:**

An acidic or alkaline formulation is bound to cause irritation on mucosal membrane and hence this parameter assumes significance while developing a mucoadhesive formulation. 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 min.

***Viscosity Study*[5]:**

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

***Estimation of drug content in formulated gels*[7]:**

Formulations containing 1 mg of drug was taken in 10 ml volumetric flask, dissolved in 0.1N sodium hydroxide made up the volume to 10 ml with 0.1N NaOH and then filtered. Absorbance values were measured at respective λmax (319 nm) for drug. Concentrations of drug were calculated from the standard calibration curve prepared in 0.1N NaOH.

***Syringeability study*[8]:**

Syringeability study was carried out by using a 22 gauze needle.

***Bioadhesion study*[9]:**

In the present study, bovine cheek pouch was used as a model mucosal surface for bioadhesion testing. The bovine cheek pouch was procured from slaughter house, then excised and trimmed evenly from the sides. It was then washed in phosphate buffer (pH 6.6) and was preserved in the same or used immediately.

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The two sides of the balance were balanced with a 5 g weight on right hand side. The bovine cheek pouch excised and washed was tied tightly with the mucosal side upwards using a thread over the protrusion in the rubber block which is covered with inert aluminum surface. The block was then lowered into the glass container, which was then filled with isotonic phosphate buffer (pH 6.6) kept at 37°C±1°C, such that the buffer just reaches the surface of mucosal membrane and keeps it moist. This was then kept below the left hand set up of the balance. The film was then glued at the border adhered to a aluminum surface hanging on the left hand side and the beam raised, with the 5 g weight removed on the right pan side. This lowered the aluminum surface along with the film over the mucosa, with a weight of 5 g. The balance was kept in this position for 8 min and then slowly water was added to the plastic container in the right pan by pipette. The addition of water was stopped as soon as the detachment of two surfaces was obtained. Weight of water was measured. The excess weight in the pan i.e. total weight minus 5 mg is the force required to separate the film from the mucosa. This gave the bioadhesive strength of the formulation in grams.

***In vitro diffusion study*[10]:**

Treatment of cellophane membrane:

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 h 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 containing 1mg of drug was taken in one cell (donor compartment) and the cell was immersed in a beaker containing 40 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, agitated by a magnetic stirrer and temperature maintained at 37±1°C throughout the study. Aliquots of 5ml were withdrawn periodically at intervals of 1 d for a period of 7 d 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.

Antimicrobial Susceptibility Test[11]:

Formulations F1 to F6 containing Metronidazole prepared and used in microbial assays. Drug equivalent to 1mg formulations used for measurement of zone of inhibition. Under aseptic conditions the formulated gels and placebo were placed on blood agar plates containing *Staphylococus aureus* and were incubated at 37°C for 24 h, after which zone of inhibition was measured. This was continued for 3 d and zone of inhibition on every 24 h interval was measured.

Sterilization method: Formulations F1 to F6 containing Metronidazole were sterilized by cold sterilization method.

RESULTS AND DISCUSSION

Periodontal gels of Metronidazole were prepared and evaluated with a view to obtain controlled release of the drug at the site of action in order to

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decrease the side effects and to increase the bioavailability of the drug at the site of action.

***Surface pH of the gel*:**

The values of surface pH were within the range of natural pH, this indicates formulations can be used without any irritation in the oral cavity. Table No. 2 showed the surface pH of all formulations.

***Viscosity Study*:**

The viscosity results showed in Table No. 2. The results indicating that formulation containing combinations of polymers are more viscous then the formulation containing only one polymers.

***Drug content*:**

All the formulations exhibit fairly uniform drug content (Table No. 2). This is because of easy and single step preparation i.e. addition of drug to the polymer solution accounted for minimal or no drug loss. F3 formulation showed more drug content (99.25 ± 0.03 %).

***Syringeability study*:**

The results of the syringeability study indicate that all the gel formulations were syringeable through 22 gauze needle. All the formulation showed viscosity values ranging from 1453.33 ± 5.77 to 1995.00 ± 0.01 dyne/ cm^2.

***Bioadhesion study*:**

The In-Vitro Bioadhesion study results are shown in Table No. 2. The bioadhesion was maximum with the F3 formulation and lowest for the F5 respectively, and all formulations showed satisfactory bioadhesion strength.

Table 2

SURFACE PH OF FORMULATED GELS, VISCOSITY STUDY OF FORMULATED GELS, DRUG CONTENT UNIFORMITY AND IN-VITRO EVALUATION OF BIOADHESION.

Formulation Code	Surface pH Mean ± SD	Viscosity of Formulated Gels Mean ± SD (dyne/ cm^2)	Drug content Mean ± SD (%)	In-vitro evaluation of Bioadhesion Mean ± SD (gm)
F1	6.12 ± 0.057	1453.33 ± 5.77	97.53 ± 0.0577	6.00 ± 0.100
F2	5.89 ± 0.057	1670.00 ± 0.00	98.23 ± 0.00577	6.13 ± 0.208
F3	5.96 ± 0.1	1866.66 ± 5.77	99.25 ± 0.033486	6.50 ± 0.100
F4	6.19 ± 0.152	1766.66 ± 2.88	97.33 ± 0.000577	2.66 ± 0.057
F5	6.32 ± 0.057	1995.00 ± 0.01	98.30 ± 0.004583	2.63 ± 0.152
F6	6.29 ± 0.057	1886.00 ± 0.00	98.53 ± 0.000577	2.73 ± 0.152

Figure 1

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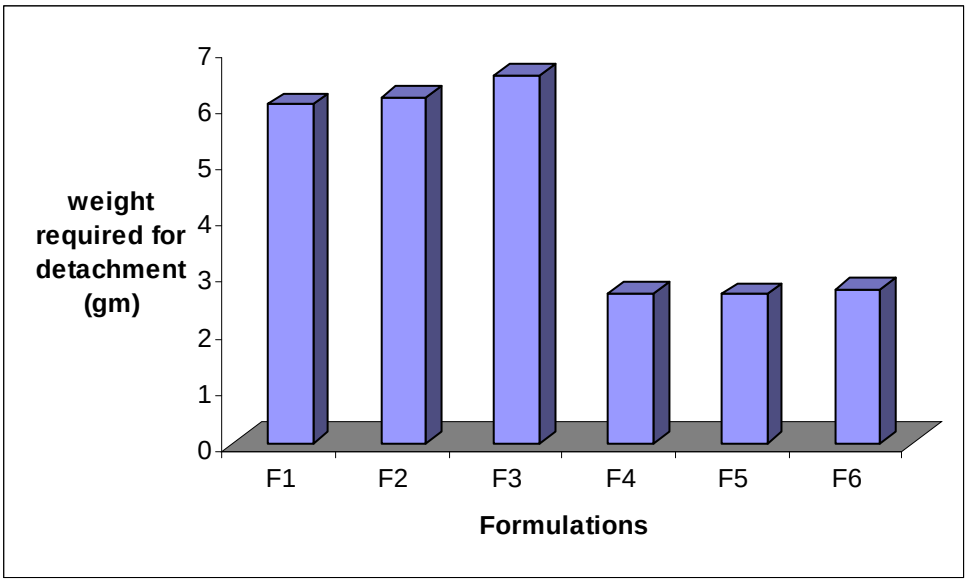
In-vitro evaluation of Bioadhesion



***In-vitro release studies*:**

In-vitro release studies were carried out by diffusion process. The result revealed (Table No. 3) that release rate of the Metronidazole was controlled for a period of time with F3 (i.e. with 3% Chitosen). The plots of the cumulative % drug release against squire root of time were found to be almost linear. Figure No. 2 showed the in vitro drug release plots of the formulations. This indicates release of the drug was controlled by diffusion controlled mechanism.

Table 3

In-vitro release profile of the Metronodazole gel formulations.

Formulation Code	% Cumulative drug release						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
F1	38.23	59	68.09	75.25	80.87	83.88	86.82
F2	36.25	57.65	64.62	68.5	75.61	79.9	82.61
F3	34.04	53.85	63.9	68.92	72.83	75.53	78.23
F4	48.81	73.55	85.61	94.59	96.72	97.72	98.72
F5	40.87	64.4	77.21	89.58	95.64	97.82	99.82
F6	42.20	66.99	85.17	90.98	95.94	97.01	98.01

Figure 2

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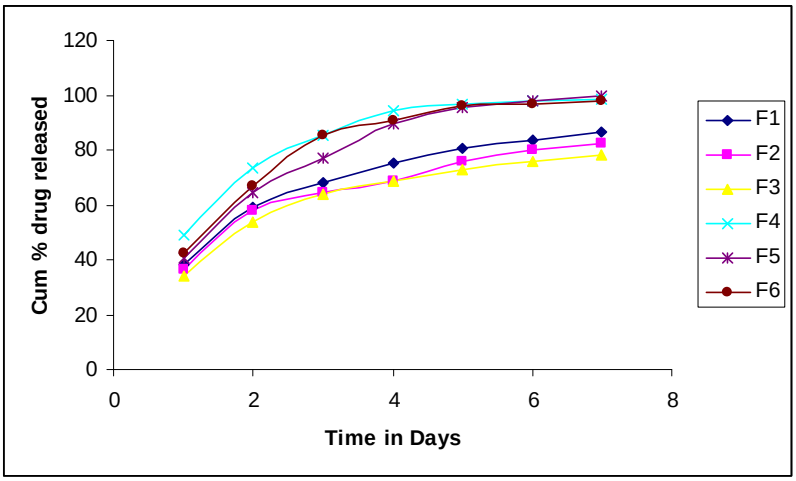
Cumulative Percentage drug released Vs Time in Days



***Antimicrobial Susceptibility Test*:**

The results of the antimicrobial studies of all the gel formulations against the *Staphylococcus aureus* indicates satisfactory zone of inhibition Table No. 4 but there was no zone of inhibition with the placebo formulation. Zone of Inhibition (mm) for formulations F1 to F6 and Placebo are shown in Plate no 1 and 2.

Table 4

Data for Zone of Inhibition of Formulated Gels of Metronidazole

Formulation Code	Zone of Inhibition (mm)		
	Day 1	Day 2	Day 3
F1	23	18	12
F2	22	16	12
F3	24	17	14
F4	25	13	10
F5	26	19	11
F6	26	20	11

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Plate No.1

Zone of Inhibition (mm) for Formulations F1, F2, F3 & Placebo

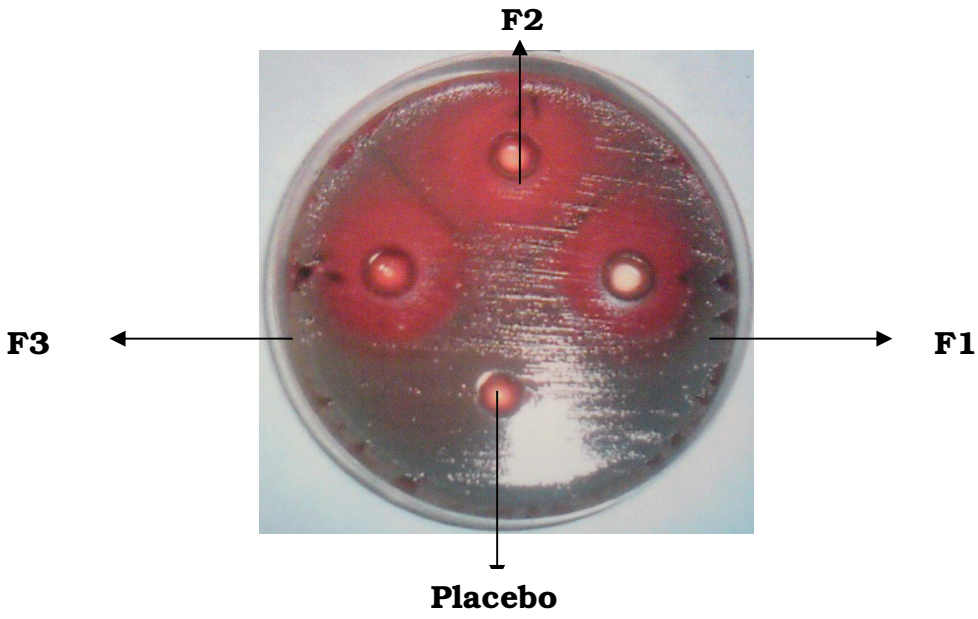
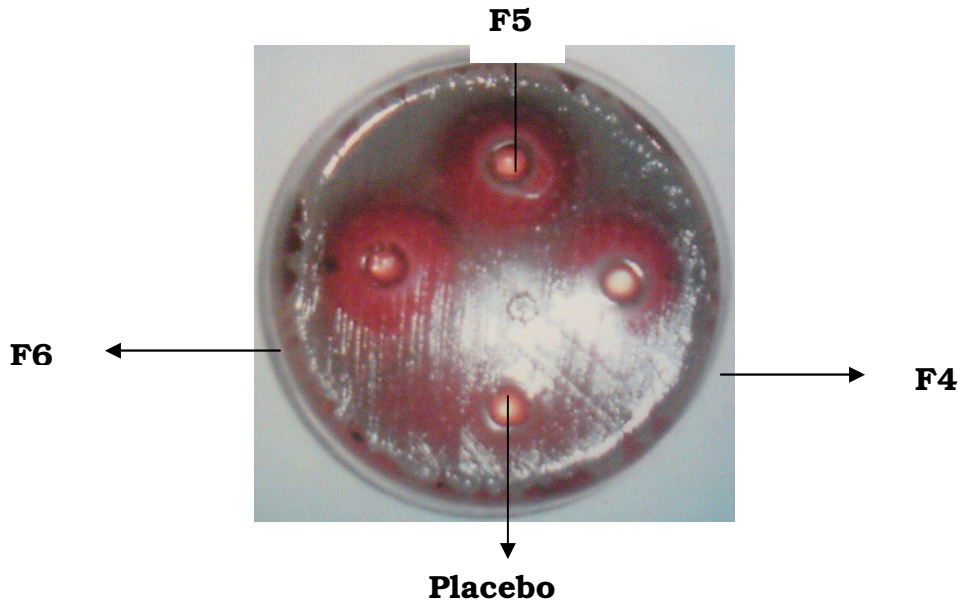


Plate No.2

Zone of Inhibition (mm) for Formulations F4, F5, F6 & Placebo



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CONCLUSION

In present study, It is concluded that Metronidazole gels can be successfully prepared using natural polymers which can be targeted in treatment of the periodontal disease and also reduce dosing frequency, increase bioavailability of Metronidazole that will result in better patient compliance with minimum side effects. Further, proper sterilization method has to be developed.

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