



## Preparation and Evaluation of Periodontal Gel of Ornidazole Using Natural Polymers

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### Abstract

Periodontal diseases are the conditions that affect the supporting structure of teeth leading to the formation of pocket due to which tooth loss occurs, for which site specific injectable drug delivery systems are gaining importance. In the present study six batches of Ornidazole gels were prepared using natural biodegradable polymers Chitosan, Xanthum gum and Locust bean gum in variable concentrations. The formulated gels were characterized for surface pH, viscosity, 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 1400 to 1975 dyne/cm<sup>2</sup>. Best formulation in terms of cumulative percent drug release along with bioadhesion was formulation F3 with 79.23% 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.

**Keywords:** Ornidazole; Chitosan; Periodontal diseases; Bioadhesion; Syringeability.

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### Introduction

Periodontitis is the common oral disease affecting many people around the world. It is defined as an inflammation and progressive destruction of the tooth-supporting structures (periodontium). This disease results from interaction between specific host defence mechanisms and dental plaque biofilms that colonize on the tooth surfaces at or below the gingival margin. The progression of periodontitis can be arrested by mechanical debridement consisting of scaling, root planing and proper oral hygiene control.[1] these treatment modalities aim to remove dental plaque and plaque-retentive factors. However, pathogenic bacteria may not be eliminated in the deep periodontal pockets due to poor access for

mechanical debridement, root anatomical complexity [2,3] and the ability of the bacteria to invade and reside in the periodontal tissues[4] or dentinal tubules.[5]

Systemic antimicrobial agents may lead to potential side effects such as development of resistant bacteria [6] and gastrointestinal intolerance.[7] These drawbacks would be markedly reduced if antimicrobial agents applied locally could be used. For the local antimicrobial agent to be useful, it must be successfully delivered to the base of the periodontal pockets at an efficacious concentration and retain in the pockets for an adequate length of time.[8] To achieve these, the sustain-released delivery drugs.[20]

It is obtained by deacylation of Chitin, is a natural, non-toxic, biocompatible and biodegradable polysaccharide suitable for applications in pharmaceutical technology. Chemically it is poly- $\beta$ -(1,4)-2-amino-2-deoxy-D-glucopyranose. It is soluble in dilute acid solutions such as dilute lactic acid and dilute acetic acid. It is insoluble at pH >6.5 and in water and most organic solvents. There is substantial variation in solubility among the Chitosan of different origin and prepared by different methods. It is used as film forming agent, gel forming agent, a dye-binder for textiles, a strengthening additive in paper and hypolipidic material in diets. It has been used extensively as a biomaterial. Owing to its immunostimulatory activities, anticoagulant properties, antibacterial and antifungal action and for its action as a promoter of wound healing is the field of surgery. [9, 10, 11 ]

Xanthan gum is the most versatile microbial exopolysaccharide. It is synthesized by the bacterium *Xanthomonas campestris*. It is soluble in both cold and hot water, but is insoluble in most organic solvents. It is used as suspensions, viscosity control, gelling agent, and mobility control. [12, 13]

Locust bean gum is extracted from the seed of *Ceratonia siliqua*. Carob bean gum occurs as a white to light yellow brown powder or granular, and is odorless or has slight odour. It is less soluble in cold water, dissolves on heating and also soluble in hot water. It has gel forming property but forms very less viscous gel alone hence it is combined with Xanthan gum or guar gum to form a viscous gel. Used as a thickener, stabilizer, emulsifier and carrier. [13]

## **Materials and Methods**

### **Materials**

Ornidazole is obtained as gift sample from Sun Pharmaceuticals, Mumbai. Chitosan, Xanthan gum and Locust bean gum are obtained from Biological E. Ltd., Hyderabad, Loba Chemie Pvt. Ltd., Mumbai, and Sigma Aldrich Pvt. Ltd., Bangalore respectively.

### **Preparation of ornidazole gel:**

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

For gel with Xanthum gum, Locust bean gum was completely dissolved in hot water, to which xanthum gum was added and dissolved using mechanical stirrer to form a gel. Ornidazole was incorporated into the formulations of required concentration by mechanical stirring.

## Evaluation of Gel

### Compatibility study of ornidazole and formulation components

The plate uniformly coated with silica gel G was activated for 1 hour by keeping in a oven. The plate was spotted with pure drug ornidazole solution in 10 ml methanol at about 2 cm apart from the bottom as standard. The six samples were spotted adjacent to the pure drug spot at a distance of 1.5 cm apart. The plate was kept in enclosed chamber saturated previously with the solvent system. The solvent was allowed to rise on the plate to a sufficient level. The distance traveled by solvent front was noted. The plate was then kept in UV chamber and the spots developed were noted and distance traveled by solute was determined. The Rf value was calculated both for the standard and the sample using the formula. Mobile phase used is Ethyl acetate : Chloroform : Methanol : Ammonia (25 : 25 : 15 : 5) and Stationary phase used is Silica gel G.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

### Surface pH of the Gel

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. [14]

### Viscosity Study

Viscosity of gels was studied on Brookfield viscometer by using spindle number 3 at 60 revolution per minute (RPMS) at constant temperature. [15]

### Estimation of drug content in formulated gels

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  $\lambda_{\max}$  (317 nm) for drug. Concentrations of drug were calculated from the standard calibration curve prepared in 0.1N NaOH. [16]

### Bioadhesion study

In the present study, bovine cheek pouch was used as a model mucosal surface for bioadhesion testing. The bovine cheek pouch is 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. The two sides of the balance were balanced with a 5 gm 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 aluminium surface. The block was then lowered into the glass container, which was then filled with isotonic phosphate buffer (pH 6.6) kept at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , 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 aluminium surface hanging on the left hand side and the beam raised, with the 5 gm weight removed on the right pan side. This lowered the aluminium surface along with the film over the mucosa, with a weight of 5 gm. The balance was kept in this position for 8 minutes 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 gm is the force required to

separate the film from the mucosa. This gave the bioadhesive strength of the formulation in grams. [17]

### ***In vitro* diffusion study**

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. Release of drug from various gel formulations was studied employing the permeation apparatus designed as described by Fites et al. 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  $37\pm 1^\circ\text{C}$  throughout the study. Aliquots of 5ml were withdrawn periodically at intervals of 1 day for a period of 7 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 317 nm. [18]

### **Antimicrobial Susceptibility Test**

Formulations F1 to F6 containing ornidazole 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 *Staphylococcus aureus* and were incubated at  $37^\circ\text{C}$  for 24 hrs, after which zone of inhibition was measured. This was continued for 3 days and zone of inhibition on every 24 hrs interval was measured. [19]

## **Results and Discussion**

### **Compatibility study of drug and polymer by thin layer chromatography:**

Formulations F1 to F6 were dissolved in 10 ml of methanol. Pure drug Ornidazole was dissolved in 10 ml of methanol. The Rf values for the pure drug ornidazole was found to be 0.76 while that of formulated gels were found to be 0.76, 0.76, 0.76, 0.75, 0.76 and 0.76 for formulations F1 to F6 respectively.

Figure. No. 1 and 2 shows the pale yellow spots obtained for pure drug Ornidazole and formulations F1 to F6. The Rf values of pure drug Ornidazole and formulations F1 to F6 have same values. It can be concluded that there was no polymer drug interaction and drug is intact.

### **Surface pH of the gel**

Table. No.1 shows the results of the surface pH value for all formulations. The values represent the mean of three replicates. They were found to be 6.0, 5.8, 5.9, 6.1, 6.2 and 6.2 for formulations F1 to F6 respectively. The values were well within the range of neutral pH. This indicates that formulation can be used and will not cause any irritation in the oral cavity.

### **Viscosity of gels**

Viscosity of gels was determined by using Brookfield Viscometer. The viscosity values in  $\text{dyne/cm}^2$  are shown in Table. No 1. The values represent the mean of three replicates. They

were found to be 1413, 1620, 1816, 1716, 1975 and 1880 dynes/cm<sup>2</sup> for formulations F1 to F6 respectively. Figure no.3 shows the results of viscosity for all the formulations. From these values it can be concluded that formulations containing combination of polymers i.e. F4, F5 and F6 are more viscous than the formulation containing only one polymer.



Figure. No. 1:



Figure. No. 2:

#### Estimation of drug content in formulated gels:

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 subtherapeutic dose or toxic dose. Drug content uniformity was also performed to ensure minimum batch to batch variations. Table. No. 1 show the values of drug content of formulated gels which were analyzed spectrophotometrically at  $\lambda_{max}$  317 nm using 0.1N NaOH. All the formulations exhibited fairly uniform drug content. 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.

**Table No. 1: Showing Surface pH, Viscosity, Drug Content, In-Vitro Bioadhesion**

Formulation Code	Surface Ph	Viscosity dynes/cm <sup>2</sup>	Drug Content	In vitro Bioadhesion
F1	6.06 ± 0.057	1413.33 ± 5.77	0.9753 ± 0.000577	5.00 ± 0.100
F2	5.83 ± 0.057	1620.00 ± 0.00	0.9823 ± 0.000577	5.13 ± 0.208
F3	5.90 ± 0.100	1816.66 ± 5.77	0.9925 ± 0.033486	5.50 ± 0.100
F4	6.13 ± 0.152	1716.66 ± 2.88	0.9733 ± 0.000577	2.16 ± 0.057
F5	6.26 ± 0.057	1975.00 ± 0.00	0.9830 ± 0.004583	2.13 ± 0.152
F6	6.23 ± 0.057	1880.00 ± 0.00	0.9853 ± 0.000577	2.23 ± 0.152

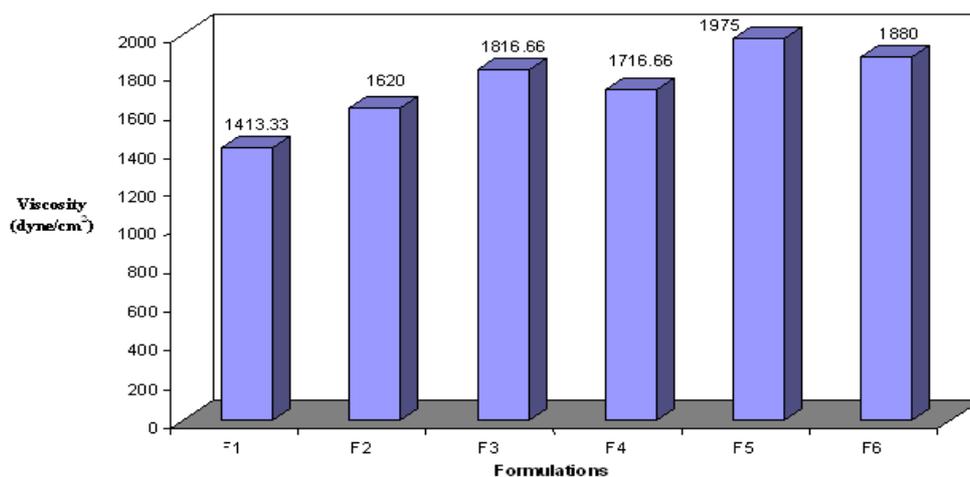


Figure No. 3. Viscosity study of formulated gels of ornidazole

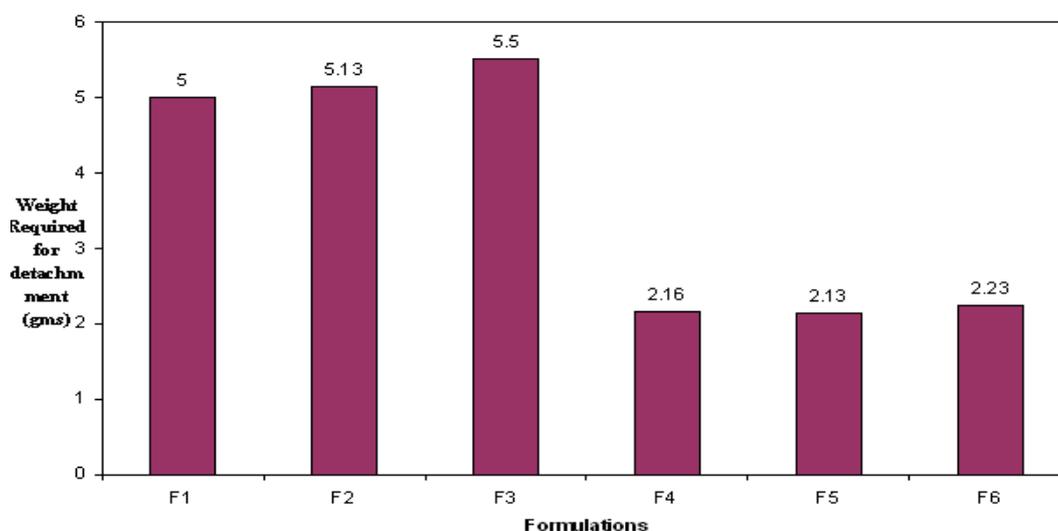


Figure No.4. In vitro Evaluation of Bioadhesion using Bovine Cheek Pouch

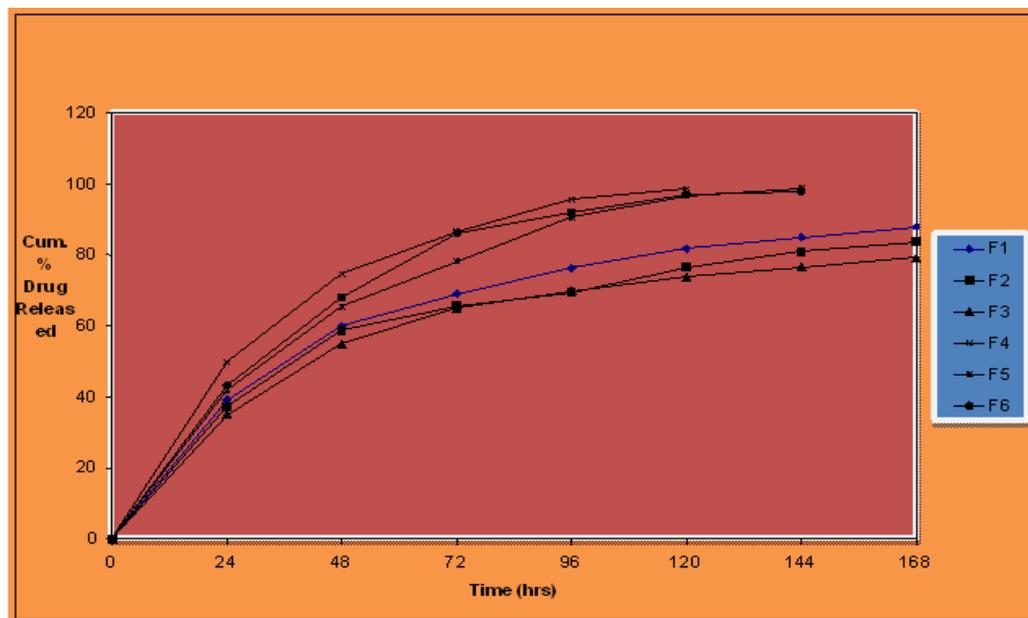
***In vitro* release studies:**

The results obtained in *in vitro* release studies were plotted in Cumulative percent drug release Vs. Time (Zero order rate kinetics). The release data obtained for formulations F1, F2, F3, F4, F5, F6 are tabulated in Table. No. 2 Figure. No. 5 shows plots of cumulative percent drug released as a function of time for different formulations.

Table. No 2: *In vitro* Drug Release of Ornidazole in PBS (pH 6.6)

S.No	Time in hrs	% Cum. Drug Released F1	% Cum. Drug Released F2	% Cum. Drug Released F3	% Cum. Drug Released F4	% Cum. Drug Released F5	% Cum. Drug Released F6
1	24	39.23	37.25	35.04	49.81	41.87	43.20
2	48	60.00	58.65	54.85	74.55	65.40	67.99
3	72	69.09	65.62	64.9	86.61	78.21	86.17
4	96	76.25	69.5	69.92	95.59	90.58	91.98
5	120	81.87	76.61	73.83	98.72	96.64	96.94
6	144	84.88	80.90	76.53	-	98.82	98.01
7	168	87.82	83.61	79.23	-	-	-

The *in vitro* release of all the six batches of gels showed an interesting biphasic release with an initial burst effect. In the first 24 hrs, drug release was 39.23%, 37.25%, 35.04%, 49.81%, 41.87% and 43.205 for F1, F2, F3, F4, F5 and F6 respectively. This was followed by a steady drug release pattern, which approximated zero order release. The mechanism for the burst release in the first few hours can be attributed to the drug which is present freely in the gel matrix.



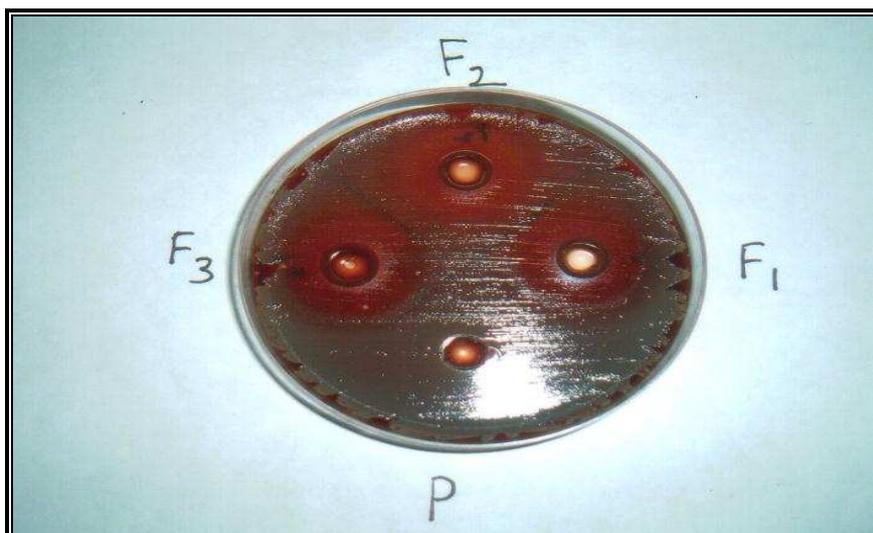
**Figure 5. Plots of Cumulative Percentage Drug Release Vs Time (Zero Order Rate Kinetics)**

#### Results for antimicrobial susceptibility test:

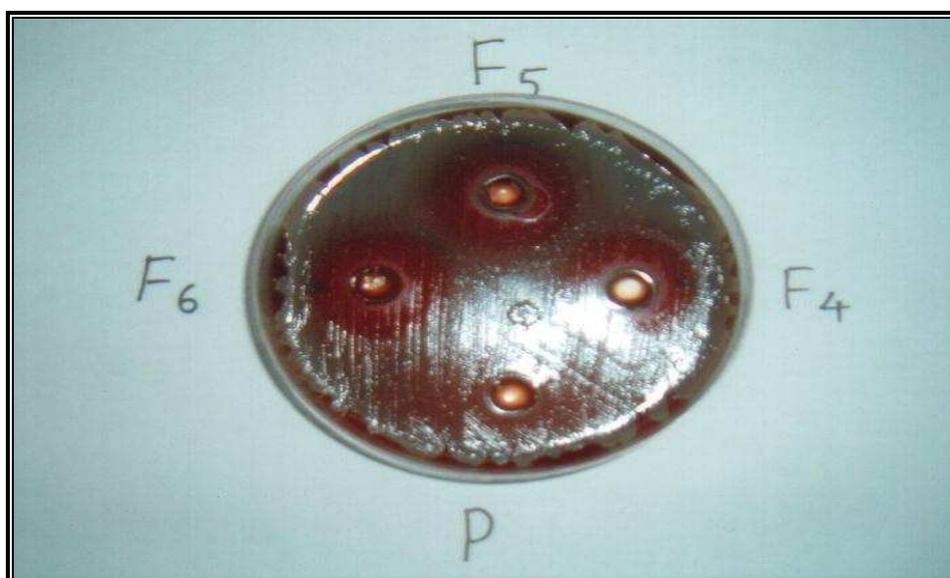
Zone of inhibition in mm was observed on first day, second day and third day which was incubated at 37°C. The data obtained was shown in Table. No. 3. Figure. No. 6 and 7 illustrates the results of antimicrobial studies against *Staphylococcus aureus* after 3 days of study. The formulation placebo P (without drug) did not show inhibition of bacterial growth. While those with drug Ornidazole showed similar pattern of activity with slight difference of zone of inhibition on all the 3 days. Results revealed that prepared formulations were found to be effective against *S.aureus* in blood agar media when compared to placebo.

**Table. No. 3: Data for Zone of Inhibition of Formulated Gels of Ornidazole**

Formulation Code	Zone of Inhibition (mm)		
	Day 1	Day 2	Day 3
F1	20	15	9
F2	19	13	9
F3	21	14	11
F4	22	10	7
F5	23	16	8
F6	23	17	8



**Figure. No. 6: Zone of Inhibition (mm) for Formulations F1, F2, F3 & Placebo (P)**



**Figure No. 7: Zone of Inhibition (mm) for Formulations F4, F5, F6 & Placebo (P)**

### **Conclusion**

The study showed that gel implants as injectable drug system can be developed by using natural polymers chitosan, xanthum gum and Locust bean gum, results indicated that the drug release occurred in a biphasic manner characterized by an initial burst effect followed by a slow release for prolonged period of time, all the formulations followed Fickian diffusion controlled and the surface pH, bioadhesive strength, viscosity study were all within the range and are suitable for use. Microbial studies showed the effect of formulations against *Staphylococcus aureus* which is depicted by zone of inhibition so we concludes that ornidazole gel implants which can be targeted in treating periodontal diseases and also reduce dosing frequency, increase bioavailability of ornidazole that will result in better patient compliance with minimum adverse effects.

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