RESEARCH ARTICLE

In-vivo, In-vitro Analysis of Formulated Colon Targeted Ornidazole Matrix Tablet

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ABSTRACT

In the existing study, using multiple natural and unnatural polymers an endeavor was made to manufacture and analyze the matrix tablet of Xanthan Gum and certain other polymers for colon-directed drug delivery systems. "The dry granulation technique was used to prepare matrix tablets, and further were coated with Eudragit S100". When weighed and analyzed for drug content, the Standard Deviation values were considerably low and negligible. As per the stipulated norms the compressive strength and friability wherein proper range. The consideration from Fourier Transforms Infrared (FTIR) and differential scanning calorimetry (DSC) determined that drug formulation is well built, and the formulated drug was homogenously diffused in the polymer matrix, which was analyzed by XRD studies. The F5 was one of the optimized tablets used to carry out *in-vitro* drug release studies in replicated gastric and intestinal fluids. F5 showed a good response to the stomach and small intestine environment and thus released the maximum amount of drug in the large intestine in 5 hours. While other formulations showed a large amount of release in the stomach and small intestine, they are not considered optimum for colon targeting. The F5 tablets were then used to carry out a Roentgenography study in Rabbit, which showed that the manufactured tablet had achieved the transit time and was well conserved for 11 hours straight. All the drugs followed non-Fickian transport. All these properties of F5 was found to be good for Colon targeting.

Keywords: Ornidazole, Colon Targeted, Eudragit S 100, Coating, Roentgenography.

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INTRODUCTION

Drug delivery from the oral route has been considered as a widely employed route of drug administration over other routes of administration that have been probed for systemic delivery of medicine in case of various dosage bureaucracies. The oral route of medication is considered to be most opportune for the management of medicine to convalescents. Dissolution and absorption of drugs from oral route of administration generally happens in belly fluid and small intestine, respectively, depending on the tablet's physiochemical attributes.

Many natural polysaccharides like xanthan gum, chitosan, locust bean gum etc: are notably liable for the advancement

Before formulating the perfect drug entity, we need to know the Human body framework for successful formulation (Table 1). Below are few aspects of being considered:

- Attributes of medications like Physiochemical, Pharmacokinetic, and Pharmaco-dynamics.
- Anatomy and physiologic traits of the gastrointestinal tract.
- Physiochemical traits and drug delivery mode of the dosage shape to be designed."[1,2]

To improve the bioavailability of the colon drug the objectives which are needed are:- drug is precisely made available at the colon, the lower dose can be administrated by control release rate, the adverse effect can be minimized and the efficacy of the colon drug is prolonged.[3] Compared to the stomach and small intestine, the vicinity of the colon is said to have less unfavorable circumstances with less diversification and strength.[4] Intestinal diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer are provincially treated by targeted drug delivery through the colon and is highly desirable in such condition.[5,6] The easiest and quick mode for targeting of API to the large intestine is to inherit slower release rates or longer releases.[7, 8]

Amoebiasis has affected about 10% of the earth's population and is convicted to cause invasive illness in about 50 million individuals and a casualty of about 100,000 among infected each year. Amoebiasis is usually found in the states where there is poor hygiene. Two species of Entamoeba have been found out from infected persons, which can cause amoebiasis. However, the percentage of people affected may differ worldwide, *Entamoeba dispar* is cause for approx 90% of infections with *E. histolytica* responsible for only 10%.[9]

MATERIAL AND METHOD:

Ornidazole, HPMC K4 M, Eudragit S100, Xanthan gum, PVP K-30,Mg.stearate, Talc and MCC.

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Drug Polymer Compatibility Studies

- Drug polymer compatibility studies were carried out using FTIR, DSC, and XRD
- The study was carried out on individual pure drugs and their physical mixture with the selected polymers.

Preparation Of Colon Targeted Ornidazole Tablets

Ornidazole, hpmc k4 m, xanthan gum, pvp k-30, and microcrystalline cellulose were taken in desired mass, amalgamated, and passed through #60 sieves, greased with magnesium stearate and talc, then was compressed into tablets by compressed on the tableting machine (rimek mini press-ii, karnavati engineering ltd.) by using 9 mm punch. The tablets were additionally coated with eudragit s 100 solutions of the coating solution. A coating solution of eudragit s 100 was then added to a mixture of isopropyl alcohol and acetone (1:1). The coating of the matrix tablet was prepared by immersing it in the coating solution, followed by a deep coating technique.[10]

Evaluation Of Colon Targeted Ornidazole Tablets

Hardness Test

Hardness designates the capacity of a tablet to endure mechanical shocks while handling. Hardness of core tablets was measured using Monsanto hardness tester. It is measured in kg/cm^2. 3 tablets were arbitrarily picked from each batch and examined for hardness.

Thickness

Thickness of Core tablets were measured using vernier calipers. Three tablets of each formulation were picked randomly determined. It is measured in mm.

Friability

Roche Friabilator is used to check the friability. Twenty tablets are taken and weighed and placed in the apparatus. Then it is rotated at 25 rpm for 4 minutes. After revolutions, the tablets were withdrawn and weighed again. The percentage friability is measured by using a formula.

$$\% F = \{1-(Wt/W)\} \times 100$$

Where, %F = Friability in per W = Initial weight of tablets Wt = Weight of tablets after revolution

Weight Variation

To study weight variation 20 tablets of each colon-specific formulation were weighed separately using an electronic balance, and the test was performed according to the official method.

Uniformity of Drug Content

The prepared Ornidazole tablet was tested for its drug content. Ten tablets of each formulation were weighed and finely powdered. The powder was taken equivalent to a dose of the drug and completely dissolved in pH 6.8 phosphate buffers, and the solution was filtered. 10 mL filtrate was suitably diluted with phosphate buffer pH 6.8 and analyzed for drug content in spectrophotometry at λ_{max}319 nm.

In-vitro Release Studies

Prepared colon targeted tablets were placed in vessels of dissolution USP type 1 apparatus (Basket method) at 100 rpm and 37 ± 0.5, containing 900 mL of 0.1 N HCl for first 2 hours then replaced by phosphate buffer (pH 6.8) solutions as dissolution medium up to 12 hrs. At calculated time intervals, 1ml samples were taken out and replaced with equal volumes of fresh buffer medium. Withdrawn test samples were filtered and analyzed by using UV-visible spectrometer.

Fourier Transforms Infrared Spectroscopy (FTIR)

The samples were crushed with KBr to make pellets under hydraulic pressure of 600 kg, the FTIR spectra were recorded between 400 and 4000 cm^{-1}(Figure 1).[11]

Differential scanning Calorimetric Analysis (DSC)

The sample were heated from 0-3000C at a heating rate of 100C/min under ARGON atmosphere using a micrometer (DSC Q20 V24.4 Build 116, TA Instruments, USA) and then thermograms were obtained. (Figure 2) [12]

***In-vitro* Drug Release in Rat Caecal Content Fluid**

Male albino rats weighing 200–250 g and maintained at normal diet were used for the study. The abdomens were made open, the caecam was traced, legated at both tips, dissected, and instantly transferred into pH 6.8 phosphate-buffered saline (PBS). The caecal bags were made open, and the constituents in it, were individually weighed, merged and then transferred in PBS to provide 4% w/v dilution.

The drug content release study was conducted using dissolution apparatus at 37 ± 0.5° and at 100 rpm using phosphate buffer of pH 6.8 containing 4% rat caecal content as a dissolution medium. Dissolution medium of pH 1.2 is taken, and dissolution is carried out for first 2 hours then followed by 7.4 pH for next 3 hours, and after the 5th hour, medium changed to phosphate buffer pH of 6.8 containing 4% rat caecal contents till completion of the study. 5 mL of sample solution was withdrawn at predetermined periods. An equal volume

Table 1: Formulation Table For Colon Targeted Ornidazole Tablet.

S.No	*Batch Number*	*Ornidazole*	*HPMC K-4M*	*Xanthan Gum*	*PVP K-30*	*Talc*	*Mg.Stearate*	*MCC*	*Total*
1	F1	100	40	60	40	5	5	100	350
2	F2	100	40	80	40	5	5	80	350
3	F3	100	40	100	40	5	5	60	350
4	F4	100	50	60	40	5	5	90	350
5	F5	100	50	80	40	5	5	70	350
6	F6	100	50	100	40	5	5	50	350
7	F7	100	60	60	40	5	5	80	350
8	F8	100	60	80	40	5	5	60	350

(All ingredients are in mg)

of fresh dissolution medium was reinstated immediately after withdrawal of the test analytes. The supernatant was filtered through a 0.45 µm membrane filter The filtrate was analyzed at 319 nm using UV Spectrophotometer. [13,14]

In-vivo Roentgenographic Study:

Healthy Rabbit weighing between 4 to 5 kg was used for the study. The optimized formulation M2 was prepared with radio-opaque barium sulphate to scan the tablet's position in the GIT. The rabbit was fasted for 12 hrs before the study and was allowed to have water ad libitum. The test formulation was administered orally using oral feeding tube. At an interval of 1, 3, 5, 7, 9, 11 & 15 hours, the X-ray photographs were taken to observe the tablet's shape, integrity, and position in the GIT (Figure 6).[15]

RESULTS

The angle of repose was found to be less than 30%; from this we can say that granules possess good flow properties. A good packing capacity of granules was indicated by Carr's index and Hausner's ratio.

The hardness was in 4.3–4.7 kg/cm^2 and friability was in the range of 0.35 to 0.60%, indicating that core tablet has good mechanical strength and drug content was in the range of 96.56% to 98.75%, and thickness was in the range of 3.4–3.5.

The drug Polymer interaction was studied by FTIR analysis and is presented in the Fig, the spectra of ornidazole showed the characteristic peaks at 3486cm^{-1}, 2933cm^{-1},18315 cm^{-1}, 1695cm^{-1}, 1545 cm^{-1}, 1296cm^{-1}.

The DCS of plain Ornidazole drug, without drug F5 tablet and drug-containing F5 tablets was performed and plotted in FIG. The drug-free tablets have shown an endothermic pinnacle at 149^{o}C, whereas drug-loaded tablets indicated an endothermic peak at 170^{o}C. The regular ornidazole has shown spiked endothermic pinnacle at 291^{o}C due to the melting point of the drug, but this peak is not seen in the drug-loaded tablets.

The XRD of pure Ornidazole, without drug F5 tablets and drug-containing F5 tablets, are presented in Figure 3. The ornidazole has shown a characteristic intense peak between 20 of 100 and 200 due to its crystalline nature in case of drug-free tablets and drug-loaded tablets, no intense peaks related notice.

The *in-vitro* drug release study was performed using dissolution rate test apparatus in gastric (0.1 N HCl pH 1.2) and intestinal fluids (phosphate buffer pH 7.4 and pH 6.8) with or without rat caecal contents. The dissolution profiles of ornidazole are given in the data is presented in Table 2 and 3.

Table 2: Post Compression Evaluation Of Ornidazole Matrix Tablet

Formulation	*Bulk Density*	*Tap Density*	*Carr's index (%)*	*Hausner Ratio*	*Angle of Repose*
F1	0.321	0.3862	15.7789	1.20	30.62^0
F2	0.318	0.389	18.25	1.22	25.64^0
F3	0.336	0.385	13.065	1.15	22.93^0
F4	0.345	0.379	8.912	1.09	21.03^0
F5	0.336	0.431	14.872	1.17	23.74^0
F6	0.335	0.406	17.48	1.21	24.77^0
F7	0.354	0.443	19.97	1.24	25.70^0
F8	0.339	0.378	14.05	1.23	30.96^0

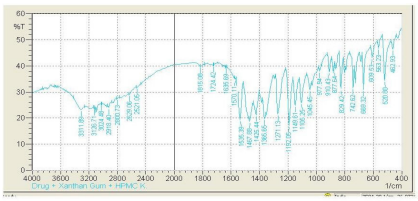


Figure 1: FTIR of ornidazole+xanthan gum+ HPMCK4m

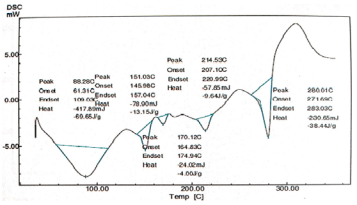


Figure 2: DSC of F5

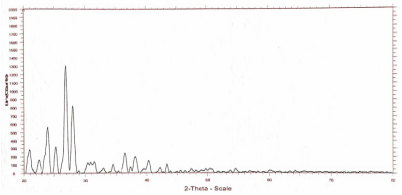


Figure 3: XRD of F5

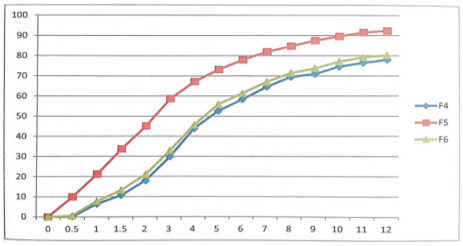


Figure 4: *In-vitro* release profile of ornidazole from F5 fromulation in gastric and intestinal simulated fluids

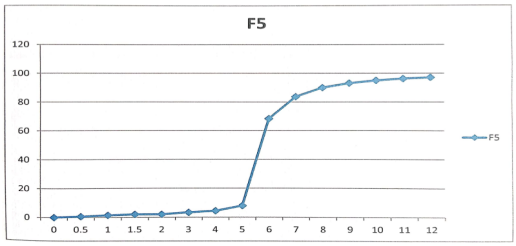


Figure 5: *In-vitro* release profile of ornidazole form F5 in gastric, intestinal and presence of rat caecal content

The Roentgenographic study by using rabbits is shown in Figures 4, 5, and 6.

DISCUSSION

The primary goal of this present work was to build up matrix tablet for colon targeted delivery of ornidazole which is used as an anti-protozoal agent in treating ulcerative colitis and moderate crohn's disease. The Ornidazole tablets were formulated by dry granulation technique using various polymers like HPMC K4M, Eudragit S 100, PVPK30, Xanthan Gum in different proportions for colon targeted drug delivery system. The outcome of the granules evaluation interpret that all the granules exhibit good flow properties, as the angle of repose values were less than 30^0. A good packing capacity of granules was symbolized by Carr's index and Hausner's ratio. The weights and drug content of all the tablets were uniforms. The hardness was in 4.3–4.7 kg/$cm^{2,}$ and friability was in the range of 0.35 to 0.60%, indicating that core tablet has good mechanical strength and drug content was in the range of 96.56% to 98.75%, and thickness was in the range of 3.4–3.5.

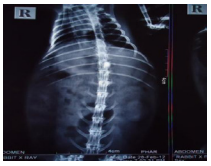
Table 3: *In-vitro* release data of ornidazole from F5 tablet in gastric and intestinal and rat caecal content fluids F5

			F5	
Time (hour)	*Square Root of time (hour)*	*Log Time (hour)*	*%drug release*	*Log% drug release*
0	0.000	0.000	0	0
0.5	0.707	-0.301	0.63	0.33
1	1.000	0.000	1.47	0.90
1.5	1.225	0.176	2.09	1.18
2	1.414	0.301	2.09	1.18
3	1.732	0.477	3.35	1.56
4	2.000	0.602	4.4	1.70
5	2.236	0.699	7.95	1.76
6	2.449	0.778	68.44	1.81
7	2.646	0.845	83.72	1.84
8	2.828	0.903	90	1.86
9	3.00	0.954	93.14	1.88
10	3.162	1.000	95.02	1.90
11	3.317	1.041	96.28	1.91

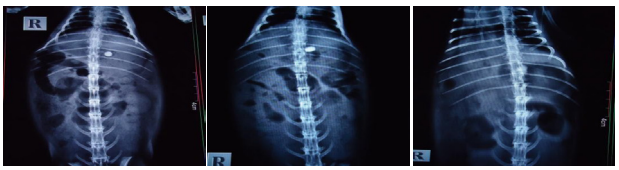
The drug Polymer interaction was studied by FTIR analysis and is presented in the Figure. The spectra of ornidazole showed the characteristic peaks at 3486 cm^{-1}, 2933 cm^{-1}, 18315 cm^{-1}, 1695 cm^{-1}, 1545 cm^{-1}, 1296 cm^{-1}. Whereas in the spectra of formulation, the same characteristic peaks related to ornidazole were noticed with very slight changes. This indicates the drug is stable, and there is no drug-polymer interaction.

The DCS of plain Ornidazole drug, without ornidazole F5 tablet and Orniadazole, loaded F5 tablets were performed and plotted in FIG. The Ornidazole-free tablets have shown an endothermic pinnacle at 149°C. at the other hand, ornidazole-loaded tablets showed an endothermic pinnacle at 170°C. The plain ornidazole has shown spiked endothermic pinnacle at 291ºC due to the melting point of the drug, but this pinnacle or peak is not shown in the drug-loaded tablets. This specifies that the drug was homogeneously dispersed in an Amorphous state The XRD of pure Ornidazole, Ornidazole free F5 tablets and Ornidazol-loaded F5 tablets are presented in FIG and ornidazole has shown typical spiked peak between 20 of 10^0 and 20^0 because of its crystalline nature whereas in case of drug free tablets and drug loaded tablets, no intense peaks related noticed. This signifies the amorphous dispersion of the drug.

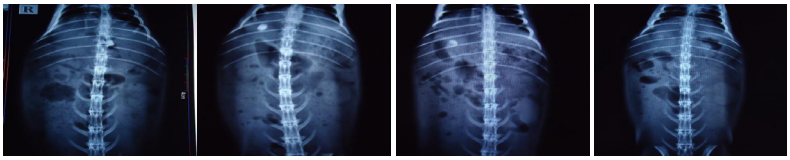
The *in-vitro* drug release study was carried out using dissolution rate test apparatus in gastric and intestinal fluids, which were simulated. The dissolution profiles of ornidazole are given in Figure 4 and 5 data are presented in Table 3 shows the drug release from tablets in simulated gastric and intestinal fluids.



AT 0 HOUR



AT 1 hour at 3 hours 5 hours



At 7 hours at 9 hours
at 11 hours at 15 hours

Figure 6: Abdominal x-ray photographs of rabbit taken at different time intervals administered with colon targeted tabelt

A 74.94%, 75.14%,80.16%,77.92%,92.3%,80.16%,81.44% and 85.63% of drug was released from F1, F2, F3, F4, F5, F6, F7 and F8 respectively at end of 12th hour. However, a 48.97%, 45.63%, 55.88%, 52.59%, 73.04%, 55.88%, 48.69%, and 62.97% drug was released from formulations respectively at the end of 5th hour in the environment of the stomach and small intestine. This indicates tablets failed to retard the drug release in the stomach and small intestine environment, the maximum amount of drug release in the upper part of the digestive system. Therefore further optimized tablets were chosen. The optimized F5 has shown minimum drug release at5th hour is 7%, and the remaining drug release was shown in the colonic region 97.11%. The work of the targeted tablet is not just protecting the drug from being released in the stomach and intestine but also releasing and maintaining the drug in the colon. Hence, *in-vitro* drug release studies were taken into consideration and carried out in the phosphate buffer pH 6.8 containing 4% rat ceacal contents. The optimized F5 formulation has been chosen for this study. The release data from tablets were fitted according to zero order release, Higuchi's equation, and Korsmeyer's equation, and the drug release phenomenon was computed according to Peppa's equation. The estimated n values alongside with the correlation co-efficient, have been displayed in Table 2. The values of n depend on the polymer concentration; the n values increases with an increase in polymer concentration. The calculated n values suggest that the phenomenon of drug release followed non-fickian transport. The Roentgenographic study showed that the tablet remained intact in the stomach and small intestine. The X-ray photographs taken at 0, 1, 3, 5, 7, 9 and 11th indicate tablets' position in the colon. The intactness of the tablet was reduced, and it was in a swollen state. The photograph taken at 15 hours of administration demonstrates the disappearance of the tablet; this is the indication for degradation of the tablet by the colonic bacteria.

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