ORIGINAL ARTICLE

Development and *in-vitro* evaluation of colon specific satranidazole tablet for the treatment of amoebiasis

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The delivery of drugs to the colon through oral route is valuable in treating diseases of the colon with the expectation to protect the drug during the transit time in the gastrointestinal tract and to allow its release only in the colon. The objective of this study was to develop colon targeted drug delivery system for satranidazole that is used in the treatment of amoebiasis. Matrix tablets containing a combination of guar gum and hydroxypropyl methyl cellulose (HPMC) K4M in different ratios were prepared by wet granulation technique followed by enteric coating with Eudragit S100. Citric acid was also added, which might further facilitate drug dissolution and absorption. All formulations were evaluated for hardness, swelling, drug content and *in-vitro* drug release studies. The results of the studies showed that colon targeted matrix tablet of satranidazole containing guar gum and HPMC K4M in the ratio proportion of 3:1 does not released drug in 0.1N HCl (pH 1.2) and small intestine (phosphate buffer, pH 7.4). When the dissolution study was continued in colonic fluids (phosphate buffer, pH 6.8), the matrix tablets released 79.21% drug while in the presence of 4% rat cecal content, it was 94.08% at the end of the 24 h. It was expected that guar gum could be degraded by colonic microflora containing anaerobic microorganism and the release may be controlled by HPMC K4M and citric acid. Studies demonstrated that orally administered Satranidazole matrix tablets can be used effectively for the delivery of the drug to the colon.

Key words: *Amoebiasis, colon specific, guar gum, hydroxypropyl methylcellulose K4M, satranidazole*

INTRODUCTION

Amoebiasis is an infection of the large intestine caused by Entamoeba histolytica, and it is mainly present in the intra-intestinal lumen. The efficient treatment of amoebiasis and other colonic infections could be achieved by targeting the drug to the colon. The delivery of drugs to the colon through the oral route is valuable in treating diseases of the colon (ulcerative colitis, amebiasis, Chron's disease, carcinomas and infections) because high local concentration, which minimizes side effects that occurs because of unnecessary systematic absorption.[1] There are several approaches, which are utilized in achieving colon targeting includes use of pH-sensitive polymer, time-dependent formulation, bacterial degradation of coating material, biodegradable polymer matrix, hydrogels and pro drugs.[2]

A colonic drug delivery system is expected to protect the drug during the transit time in the gastrointestinal tract and to allow its release only in the colon. The various approaches that have been studied for targeting orally administered drugs to the colon include the use of pH-sensitive polymers,[3] time-dependent dosage forms[4,5] and the use of carriers degraded by enzymes produced by colonic bacteria.[6] On the basis of these approaches, the use of materials that are degraded by the colonic microflora has been found to be the most promising because of their site specificity.[6,7] These polymers shield the drug from the environment of the stomach and small intestine and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by micro-organisms or degradation by enzymes.[8,9] Polysaccharides such as chitosan, pectin,

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inulin and guar gum have been explored for their potential in colon-specific drug delivery.[10]

Satranidazole, a 5-nitroimidazole substituted at the second position and has been found to be more active against aerobic, microaerophilic and anaerobic bacteria than metronidazole.[11] When directly targeting the drug to colon, the maximum concentration of drug reaches and increases the residence time of drug in the colon. Because of many of the protozoan especially *Entamoeba histolytica* remains confined in the large intestine, which necessitates high intracolonic drug concentration.

Guar gum is being used to deliver drugs to colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine. The anaerobic bacteria that are responsible for the degradation of guar gum in the colon are *Bacteroides* species *(Bacteroides fragilis, Bacteroides ovatus, Bacteroides variabilis, Bacteroides uniformis, Bacteroides distasonis and Bacteroides thetaiotaomicron*). The gelling property retards release of the drug from the dosage form as well as it is susceptible to degradation in the colonic environment.[12] Furthermore, its high molecular weight is responsible for metabolizing it in the large intestine due to the presence of microbial enzymes.[13,14]

Eudragit S is a co-polymer of methacrylic acid and its methyl esters and is soluble above pH 7. The use of such polymer based on the view that the pH of the gastrointestinal tract does not exceed 7 until the distal ileum is reached.[3]

The present study was undertaken to investigate the formulation of colon specific matrix tablet using inexpensive, naturally and abundantly available polysaccharide, guar gum as a matrix former and hydroxypropyl methylcellulose (HPMC) K4M as a release controlling polymer in the colon, citric acid to facilitate the drug solubility in the colon[15] and coating the tablet with enteric coating with Eudragit S100. The release profiles of these tablets were further compared in the presence and absence of rat cecal contents.

MATERIALS AND METHODS

Materials

Satranidazole was obtained as a gift sample from Alkem Laboratories Mumbai, India. HPMC K4M was received from Zim Laboratories, Nagpur. Eudragit S100 was procured as a gift sample from Degusssa (Darmstadt, Germany). Guar gum was purchased from Pure Chem Lab, Mumbai, citric acid from Merck Chemical, Mumbai, dibasic calcium phosphate from S. D. Fine Chemicals Limited, Mumbai and maize starch from Loba Chemicals, Mumbai. All other solvents and reagents used were of analytical grade.

Methods

Formulation of tablet by wet granulation

Matrix tablets of satranidazole were prepared by the wet granulation technique. The composition of different matrix formulations used in the study is given in Table 1. All formulations contain previously sieved guar gum and HPMC K4M in the ratio of (1:1, 2:1, 3:1, 4:1) respectively and vice versa. After blending and granulation with 10% starch paste, the wet mass was passed through sieve no. 18 and the granules were dried at 45°C for 15-20 min. Dry granules were again passed through sieve No. 18. The lubricated granules were compressed using 12 mm flat punch on rotary compression machine.

Composition of coating solution

The composition of coating solution is given in Table 2. Eudragit S100 was dissolved in iso propyl alcohol and stirred until the uniform mixture is formed. To the solution 1% plasticizer was added and stirred again to produce uniform mixture. The coating was carried out by using a conventional coating pan.

Enteric coating of tablet

The prepared concave tablets were loaded to a coating pan

Table 2: Composition of coating solution

Composition	%
Eudragit S100	5% w/v
Propylene glycol	1% v/v
Isopropyl alcohol	q.s.

Table 1: Formulation of colon specific matrix tablet

Code	Drug SZ (mg)	Guar gum (mg)	HPMC K4M (mg)	Dibasic calcium phosphate (mg)	Citric acid (mg)	Starch (mg)	Talc (mg)	Mg stearate (mg)	Total weight (mg)
S1	300	225	-	60	75	75	7.5	7.5	750
S2	300	112.5	112.5	60	75	75	7.5	7.5	750
S3	300	150	75	60	75	75	7.5	7.5	750
S4	300	168.75	56.25	60	75	75	7.5	7.5	750
S5	300	180	45	60	75	75	7.5	7.5	750
S6	300	75	150	60	75	75	7.5	7.5	750
S7	300	56.25	168.75	60	75	75	7.5	7.5	750
S8	300	45	180	60	75	75	7.5	7.5	750

HPMC: Hydroxypropyl methylcellulose K4M

and dried for few minutes with the help of dryer. The tablets were coated by spray coating using a spray gun. The pan speed was kept at 15 rpm and hot air was blown (40°C) over the tablets using the dryer. The tablets were coated until it attains predetermined weight i.e., the % coating becomes 5%. Finally, coated tablets were dried at 40°C for 10-15 min.

Evaluation of colon targeted matrix tablets of satranidazole

Thickness of tablets

The thickness of six tablets was measured using a vernier caliper. The extent to which the thickness of each tablet deviated from ± 5% of the standard value was determined.

Hardness

Hardness of the tablet was determined by Monsanto hardness tester. Six tablets from each formulation batch were selected and evaluated and the average value with a standard deviation was recorded.[16]

Friability

Friability of tablets was performed in a Roche friabilator. Ten tablets were weighed together and then placed in the chamber. The friabilator was operated for 100 revolutions and the tablets were subjected to the combined effects of abrasion and shock because the plastic chamber carrying the tablets drops them at a distance of six inches with every revolution. The tablets are then dusted and re-weighed.[17]

Weight variation

Twenty tablets were selected at random and average weight was determined. Then, individual tablets were weighed and the individual weight was compared with the average weight. The percentage deviation was calculated and checked for weight variation. Using this procedure weight variation range of all batches of formulations was determined and recorded.[17]

Uniformity of drug content

From each formulation batch, 10 tablets were weighed. Tablets were triturated in a mortar and finely powdered. Quantity equivalent to 25 mg of satranidazole was accurately weighed and transferred to a 100 ml volumetric flask and stirred in 0.1 N HCl for 10 min. Finally, the volume was made up to the mark with 0.1N HCl solution. The solution was then filtered through Watmann filter paper (# 41), the filtrate was appropriately diluted to get a final concentration of 15 μg/ml of satranidazole. Absorbance of these solutions was measured at 319 nm.[18]

Swelling behavior of tablets

One tablet from each formulation was randomly selected, weighed individually (W1) and placed separately in Petri dishes containing 10 ml of water. After each hour, the tablets were carefully removed from petri dishes and excess water was removed using filter paper.[19] The swollen tablets were reweighed (W2) and swelling index of each tablet was calculated using the following equation and expressed in percentage

$$\text{Swelling index}\,(\%) = \frac{\text{Wt. of swollen tablet - Initial wt. of tablet}}{\text{Initial wt. of tablet}} \times 100 \qquad (1)$$

In-vitro dissolution studies

The drug release studies were carried out using USP dissolution test apparatus[20] at 100 rpm and 37 ± 0.5°C temperature using 900 ml. About 0.1 N HCl as a dissolution medium in the first 2 h of study as the average gastric emptying time was estimated at 2 h. Dissolution medium 5 ml was withdrawn after 2 h to determine the drug release. The volume withdrawn with replacing with fresh media and was accounted during calculation of cumulative percentage drug release. The amount of drug release was analyzed by double beam UV spectrophotometer at maximum wavelengths of 319 nm. At the end of 2 h, the dissolution media was replaced with 900 ml of phosphate buffer (pH 7.4) and drug release study was continued for another 3 h. (i.e., total 5 h) as the average small intestine transit time is about 3 h. The samples were withdrawn at regular time intervals and correspondingly replaced with fresh media. At the end of 5 h, the dissolution media was replaced with 900 ml of phosphate buffer (pH 6.8) and drug release study was continued for next 19 h. The removed samples were diluted up to 10 ml of respective medium. The amount of drug release was analyzed spectrophotometrically at λmax of 319 nm.

In-vitro dissolution studies in the presence of 4% w/v rat cecal content

After approval of the protocol from scrutiny committee of Institutional Animal Ethical Committee (Registration no. 652/02/a/CPCSEA), animals were used in the investigation. The susceptibility of the matrix tablet to the enzymatic action of colonic bacteria was assessed by performing the drug release in medium contacting rat cecal content. Cecal material was collected from male Albino rats weighing 200-250 g, maintained on a normal diet. The cecal enzyme production was induced by giving orally 1 ml of 2% w/v dispersion of guar gum for 7 days (administered directly into the stomach using Teflon tubing). Thirty minutes before the commencement of drug release studies, four rats were sacrificed and abdomen was opened, the cecal was isolated, ligated at both ends, cut loose and immediately transferred into phosphate buffer (pH 6.8) previously bubbled with nitrogen (N_2). The cecal bags were opened and their contents were individually weighed, pooled and then suspended in phosphate buffer to give a final cecal dilution of 4% w/v. The dissolution study was continued using 100 ml of the above made rat cecal media after the 5th h. This is done with slight modification in the experimental setup of the USP dissolution test apparatus. A beaker of 150 ml capacity containing

100 ml of phosphate buffer with rat cecal content was placed suitably in the dissolution vessel having a temperature maintained at 37 ± 0.50°C, which in turn was kept in the water bath of the apparatus. The study was continued from 5 h to 24 h and samples were withdrawn at regular intervals for analysis and each time replaced with fresh phosphate buffer media containing rat cecal material bubbled with N_2.[21] The withdrawn samples were diluted with 50 ml of phosphate buffer and centrifuged. The supernatant was filtered through a bacteria proof filter and the filtrate was analyzed for satranidazole content using a double beam UV spectrophotometer at λmax of 319 nm.

RESULTS AND DISCUSSION

The results of evaluation of properties of coated and uncoated tablet are given in Tables 3 and 4.

The hardness of uncoated tablets was found in the range of 4.8 ± 0.05 to 5.2 ± 0.08 kg/cm^2. The hardness of coated tablets was found in the range of 6.9 ± 0.08 to 7.4 ± 0.16 kg/cm^2. Hardness values were satisfactory and indicated good mechanical strength of tablets. The thickness of uncoated tablets was found in between 3.93 ± 0.06 mm and 4.13 ± 0.06 mm and for coated tablet thickness found to be 4.07 ± 0.06 mm to 4.26 ± 0.06 mm and thickness of coating found to be in between 0.03 ± 0.11 mm and 0.23 ± 0.058 mm. All tablets showed loss of less than 1% in weight, which was considered acceptable. The average weight of the tablet was 750 mg with weight variation (750 mg ± 5%). Thus, all formulations were found to be complying with the standards given in the IP. Uniformity of weight test for all formulations was carried out using the procedure described in the methodology section and the results are shown in Table 3. Good and uniform drug content (>99) was observed within the batches of different tablet formulation. Swelling index was found to be highest for tablet from batch S1. It shows that formulation containing highest percentage of guar gum swells more than other formulations.

Swelling behavior of tablets

The matrices % swelling increases at the beginning attains a maximum and then declines as can be seen in Figure 1. The matrices behavior can be described in a natural hydration process. Hydrophilic matrices in contact with water swell and increase their volume and weight due to water diffusion through the matrix. The polymer chains continue the hydration process and the matrix gain more dissolution medium.

In-vitro dissolution studies

The ability of guar gum matrix tablet of satranidazole to remain intact in the physiological performed as per the procedure described in the methodology section. Environment of the stomach and small intestine was assessed by conducting drug release studies under condition mimicking mouth to colon transit.

The release profiles of satranidazole from coated tablets were evaluated in two types of dissolution media. The first phase

Table 3: Evaluation of physicochemical parameters of uncoated tablets

Parameters Batch	Hardness (kg/cm^2)* (±SD)	Thickness (mm)* (±SD)	% friability	% Wt variation (mg) (*n*=20)	Drug content (%)
S1	5.1±0.12	4.06±0.05	0.40±0.13	749.85±0.98	99.19±0.34
S2	4.9±0.17	4.03±0.15	0.62±0.96	750.55±0.88	99.63±0.83
S3	4.8±0.05	4.13±0.06	0.54±0.75	750.90±0.79	100.14±0.91
S4	5.2±0.08	4.00±0.20	0.67±0.75	749.85±0.74	100.21±0.43
S5	5.1±0.04	3.96±0.11	0.67±0.82	749.86±0.81	99.48±0.77
S6	4.9±0.17	4.13±0.06	0.52±0.60	750.00±0.97	99.78±0.58
S7	5.1±0.12	4.06±0.12	0.40±0.26	749.90±0.85	100.29±0.33
S8	5.2±0.05	3.93±0.06	0.66±0.79	750.30±0.73	99.70±0.67

*n=3

Table 4: Evaluation of physicochemical parameters of coated tablets

Parameters Batch	Hardness* kg/cm^2±SD	Thickness* mm±SD	Diameter* cm±SD	Thickness of coating* mm±SD
S1	7.1±0.04	4.23±0.05	1.23±0.06	0.17±0.12
S2	7.0±0.21	4.16±0.06	1.26±0.0.5	0.13±0.21
S3	6.9±0.08	4.23±0.11	1.23±0.11	0.10±0.1
S4	7.3±0.04	4.07±0.06	1.20±0.10	0.07±0.15
S5	7.2±0.09	4.10±0.10	1.26±0.06	0.13±0.05
S6	7.0±0.12	4.26±0.06	1.23±0.05	0.13±0.06
S7	7.4±0.08	4.10±0.20	1.13±0.06	0.03±0.11
S8	7.4±0.16	4.16±0.06	1.16±0.12	0.23±0.058

*n=3

consist of the use of 0.1 N HCl (pH 1.2) as dissolution media and in the second phase consist of phosphate buffer (pH 7.4) as dissolution media to simulate the pH conditions in the stomach and intestine, the third phase was with the simulated colonic fluid of phosphate buffer (pH 6.8). The second type of the dissolution study was done with phosphate buffer containing 4% rat cecal content in anaerobic condition in the final phase of the dissolution study; keeping the first two phases same with the same dissolution medias. Phosphate buffer (pH 6.8) was used to represent the environment after the system entered the ascending colon, where the disaccharide was degraded into organic acids by colon bacteria. The functionality of the enteric coating is to maintain the integrity of the system in the stomach. The comparative release of all batches in the presence and absence of 4% rat cecal content showed in Figures 2 and 3.

Based on release studies, batch showing best release was selected as best formulation batch and its release in the presence and absence of rat cecal content shown in Figure 4.

The coated tablets remained intact in 0.1 N HCl (pH 1.2) and no release were observed with all the batches. In the second phase of the dissolution study, the enteric coat got dissolved but and there was not more than 6% of drug release seen with all the batches of formulation. In the final phase of the dissolution study with simulated colonic fluid as the dissolution media, the drug release was 34% to 45% in the phosphate buffer (pH 6.8) and 58% to almost 71% drug release were found in the phosphate buffer containing 4% rat cecal content as colonic microflora at the end of 12 h. It can be seen that there was a very significant difference when the medium was changed from first to the second study in the final phase. In batches from S1 to S8, the drug release in phosphate buffer (pH 6.8) was 63-79% and from S1 to S8 79-94% in the presence of colonic microflora at the end of 24 h. The highest *in-vitro* dissolution profile at the end of 24 h was shown by batch S4 containing guar gum and HPMC K4M in the ratio of (3:1) respectively was found to be (79.21%) followed by batch S5 containing guar gum and HPMC K4M in the ratio of (4:1) respectively was found to be (75.96%) while

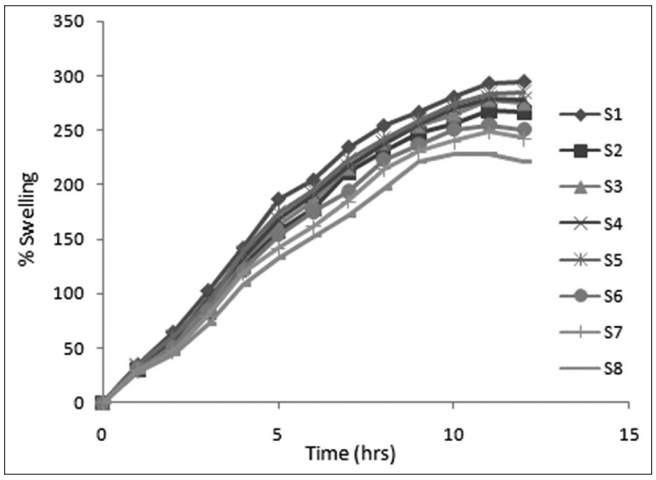

Figure 1: Plot of percent water uptake (% swelling) by tablets from batches S1 to S8 as a function of time

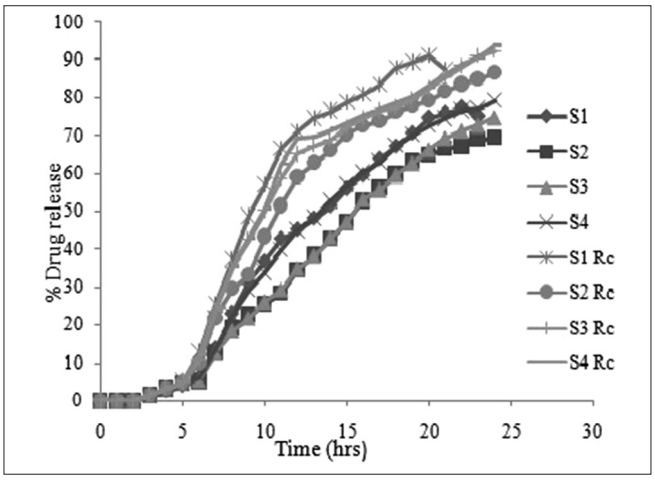

Figure 2: Comparative % release of satranidazole from S1 to S4 batches in absence and presence of 4% rat cecal content

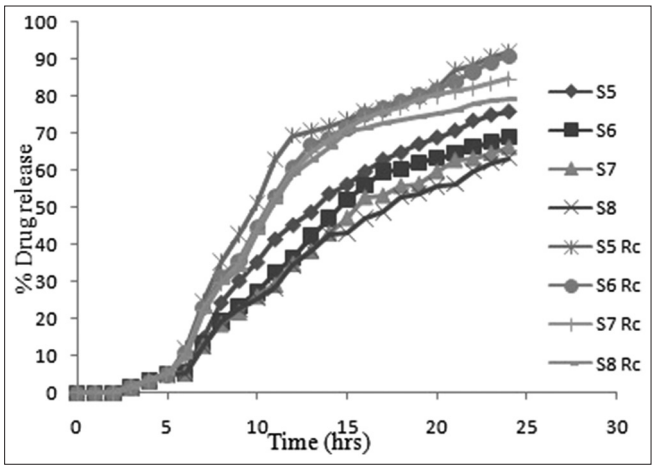

Figure 3: Comparative % release of satranidazole from S5 to S8 batches in absence and presence of 4% rat cecal content

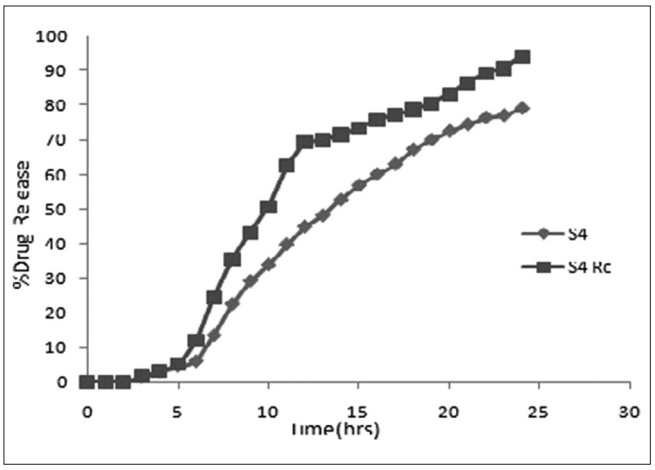

Figure 4: Comparative % release of satranidazole from best formulation batch (S4) in absence and presence of 4% rat cecal content

in the presence of rat cecal content batch S4 release highest amount of drug (94.08%) and their comparative release in both dissolution media shown in Figure 4. From the *in-vitro* dissolution studies in the presence of rat cecal content, it was found to be that the drug release increased in the presence of 4% w/v rat cecal content and the colon targeted matrix tablet containing guar gum and HPMC K4M in the ratio (3:1) released 94.08% of satranidazole. It may be due to the presence of colonic bacteria, which act on the guar gum and digest it. Therefore, released maximum quantity of satranidazole in the colon and retard the drug release in the environment of the stomach and small intestine. During stability studies, there were no significant variations in the appearance, hardness, friability and drug content and *in-vitro* dissolution studies.

CONCLUSION

The present study was aimed at developing colon targeted drug delivery system of satranidazole. Guar gum when used along with HPMC K4M in the ratio of (3:1) in an all 30% total polymer concentration shows better results and releases 94.08% of drug in the presence of colonic bacteria. Coating with Eudragit S100 exhibited more capacity to protect the drug from being released in the upper parts of the gastrointestinal tract. It indicated that the formulations succeed in targeting the drug to colon and hence can be considered better for site specificity. Selection of better dissolution model helped in understanding the release pattern. From the data, it can be concluded that polysaccharides alone cannot be used either for targeting the drug to the colon or for sustaining or controlled release of the drug.

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