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FORMULATING COLON SPECIFIC DELIVERY SYSTEM FOR METRONIDAZOLE USING PROBIOTICS AS MEDIATORS OF COLONIC TARGETING

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ABSTRACT
Matrix tablets of metronidazole were prepared using chitosan as the polymer and Rhamno G in varying quantity as the probiotic to assist the targeted release of the drug in the colonic microflora. The tablets were prepared using wet granulation method and were evaluated for precompression and post compression parameters. The angle of repose was found to be in the range of 25°71' to 27°41'. The bulk density value ranged from 0.42 to 0.46 g/cm^3 while the tapped density value ranged from 0.48 to 0.52 g/cm^3. The granules prepared in the present work were found to have the Carr's Index between 15.68 to 11.53 % and the Hausner's ratio was found to be ranging from 1.186 to 1.130. The thickness of all the tablets was found to be 7.2-7.4 mm while the hardness was 6 kg/cm^2. The friability of all the formulations was less than 1% while the weight variation was 1.1 to 1.5 %. The drug content ranged from 97.9 to 98.7 % in the formulations. The tablet formulations were investigated for the *in vitro* drug release in absence and presence of rat caecal content and it was found that the release of drug from the tablets was affected by the presence of probiotic. The rat caecal content was able to increase the drug release from all the formulation.

KEYWORDS: Metronidazole, colonic delivery, probiotic, microflora, gastrointestinal tract, tablet.

INTRODUCTION
Diseases like Crohn's disease (CD), ulcerative colitis (UC), or the irritable bowel syndrome (IBS) have been known to have wide impact on the colon.[1] Frequently administered drugs for managing these conditions include metronidazole, dexamethasone, sulfasalazine among others. All these drugs require to be delivered to the colon without showing a release in the upper portions of the gastrointestinal tract (GIT).[2] A very convenient approach for colon targeted delivery is administrating the drug through the rectum but it possesses the major disadvantage of patient incompliance.[3] The most patient compliant route for drug administration is the oral route and several methods have been investigated for colonic delivery of drug through the oral route like prodrug concept[4], matrix based systems[5], timed release techniques[6], bioadhesion[7], multiparticulate systems[8], polysaccharide based systems[9] and enteric coating systems[10] have been explored for colonic delivery.

Polysaccharides such as chitosan, guar gum, pectin, etc., are commonly employed as release rate-controlling components in colon-targeted dosage forms. These polysaccharides are known to be resistant to gastric and intestinal enzymes, but are metabolized by anaerobic bacteria in the colon.[11]

Polysaccharide-based delivery systems have several advantages and are therefore becoming a popular option for colon-specific delivery of drugs. Some of the advantages with polysaccharide use include availability, easy modifications, stability, safety, and biodegradability.

Probiotic supplements (Bifidobacteria spp and Lactobacilli) are known to improve resistance to gut infections by inhibiting the growth of harmful bacteria, to reduce cholesterol levels, improve the immune response and produce vitamins.[12]

Various probiotic-containing preparations like Rhamno G (*Lactobacillus rhamnosus GG*) are available in the market in spore form. The objective of the present work was to combine the colon specific potential of probiotic formulation Rhamno G and chitosan for designing matrix tablet based delivery system for colon targeting.

MATERIAL AND METHODS
Metronidazole was obtained as generous gift sample from Medibios Laboratories, Tarapur. Talc, magnesium stearate, lactose monohydrate, and chitosan were purchased from Oxford Fine Chemicals LLP Mumbai. Rhamno G capsules were purchased from local Pharmacy store of Bhopal. All other chemicals and

reagents used were of analytical grade and used as obtained.

Preformulation Studies[13]

Organoleptic Evaluation

The color, odor and taste of the obtained drug sample were observed with the help of the sensory organs.

Solubility (at room temperature)

Solubility was determined in different solvents like water, HCl, ethanol and acetone.

Identification Test

FT-IR spectrum of the sample of metronidazole was obtained and examined for the presence of characteristic peaks and matched with that of the reference spectra in databases for confirmation of the identity of the drug.

Melting point determination: Melting point was determined by open capillary method by placing a small quantity of powder into capillary tube and placed in the melting point apparatus. The temperature of the apparatus was gradually increased and the temperature at which the powder started to melt and the melting temperature was recorded.

Calibration Curve in PBS pH 7.4

Accurately weighed 10 mg of metronidazole was taken in 100 mL volumetric flask and dissolved in water and the volume was made up to the mark with PBS pH7.4. The solution was suitably diluted with PBS pH7.4 to obtain solutions of 10-50 ppm. The calibration curve was prepared by measuring the absorbance of the solutions at 340 nm using UV spectrophotometer[14].

***In vitro* digestion of chitosan by probiotic**

A dispersion of chitosan was prepared by dispersing 1 g of chitosan in 100 mL distilled water. To the slurry was emptied the content of one capsule of Rhamno G and the mixture was incubated at 37°C in incubator overnight. The pH and viscosity of the dispersion was measured at various time intervals. A dispersion of chitosan without probiotic was also studied as control using the same procedure.[15]

Formulation of matrix tablet of metronidazole using chitosan

The matrix tablets of metronidazole were prepared by wet granulation method.[12,15] Chitosan, metronidzole, lactose, talc and magnesium stearate were passed separately through sieve number 60. Metronidazole, lactose and chitosan were mixed together (Table 1) and water was added to the blend. The wet mass was passed through sieve number 20 and the granules were dried at 50°C in oven for 1 h. The dried granules were passed through sieve number 16. Magnesium stearate and talc were added to the granules and blended for 5 min in a polybag. The lubricated granules were compressed using single punch tablet press.

Formulation of matrix tablet of Metronidazole using chitosan-probiotic mixture

The matrix tablets of metronidazole were prepared using chitosan (40% of tablet weight) using the method above. The probiotic was added in two portions: half prior to granulation and the other half prior to final blending of the mixture.

Table 1: Composition of the tablet formulation.

Ingredient	Quantity of each ingredient per tablet in mg				
	F1	F2	F3	F4	F5
Metronidazole	100	100	100	100	100
Chitosan	100	150	200	250	300
Lactose	285	235	135	75	15
Magnesium Stearate	5	5	5	5	5
Talc	10	10	10	10	10
Rhamno G	-	-	50	60	70
Weigh of tablets	**500**	**500**	**500**	**500**	**500**

Micromeritic features of granules[16]

Angle of repose, Carr's Index, Bulk density, Tapped density and Hausner's ration were determined to assess the flow ability of the prepared granules.

Evaluation of the prepared tablets[17]

Thickness

The thickness of 20 tablets from each batch was measured using a digital vernier caliper.

Weight variation test

20 tablets were weighed and the average weight was calculated. Each of these tablets was individually weighed and the difference from average weight was calculated. The percent weight variation was calculated.

Friability test

The friability of the formulations was performed using a Roche type friability test apparatus. Twenty tablets were initially weighed ($W_{initial}$) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes, that tablets were collected, dedusted and reweighed. The tablets were weighed again (W_{final}). The percentage friability was then calculated by the formula

$$\% \text{ Friability} = \frac{W_{initial} - W_{final}}{W_{initial}} X\ 100$$

Hardness test

The hardness of the formulated tablets was tested using Monsanto type hardness tester. Three tablets from each batch of formulation were taken and the force required to break the tablets was directly measured using hardness tester.

Drug content

Five tablets were weighed to determine the average weight and powdered using mortar-pestle. An amount of powder equivalent to 10 mg of drug was transferred in 100 mL of PBS pH 7.4. 1 ml from this stock solution was withdrawn and diluted up to 10 mL with PBS pH 7.4. Absorbance of the resulting solution was measured at 340 nm using UV spectrophotometer and the concentration was determined using the calibration curve.

In vitro release study

In vitro dissolution studies for all matrix tablet formulations were performed by using basket type dissolution test apparatus using phosphate buffer pH7.4 as the dissolution medium. The tablet was placed in the basket and the dissolution study was carried out for 12 h. At predetermined intervals, 1 mL of the dissolution media was pipetted out and was diluted to 10 mL using PBS pH 7.4. Absorbance of the solution was recorded at 340 nm using UV visible spectrophotometer.

In vitro release studies in presence of rat caecal content

Preparation of rat caecal content

Wistar rats weighing 150-200 g were used to obtain the caecal content. The rat were administered with 4 mL of 1% w/v of dispersion of chitosan in water for 7 days. Thirty minutes prior to the start of study, 3 rats were killed by spinal traction, the abdomen was dissected open and immediately transferred to pH 6.8 phosphate buffer. The caecal bag was opened and its content was weighed, homogenized, and suspended in phosphate buffer pH 6.8 to obtain a concentration of 4% w/v of caecal content.[18]

In vitro dissolution studies

The drug release for all the formulations was carried out in phosphate buffer pH 7.4 with rat caecal content (4%w/v). At fixed time intervals, 1 mL of the dissolution media was pipetted out and its volume was made up to 10 mL using PBS pH 7.4. Absorbance of the solution was observed at 340 nm using UV spectrophotometer.

RESULTS AND DISCUSSION

The physical characterization of the drug was performed according to the reported procedure and the results obtained were compared with that of the standard specifications (Table 2).

Table 2: Prefromulation parameters of metronidazole.

Oganoleptic features	Melting Point	Solubility
White, odourless, powder	157-159 °C	Soluble in water, ethanol, 0.1N HCl; slightly soluble in chloroform

FTIR spectral studies

The IR spectrum of the drug sample of metronidazole was obtained and the stretching vibrations due to O-H (3318 cm^{-1}), C=O (1770 cm^{-1}), C-H (1458 cm^{-1}), aromatic C=C (1344 cm^{-1}), C-N (1307 cm^{-1}), C-O (1280 cm^{-1}), C-N aromatic amine (1243 cm^{-1}) and bending vibrations due to N-H (1580 cm^{-1}) and C-H (1417 cm^{-1}).

Calibration curve of metronidazole

The absorption maximum of metronidazole in PBS7.4 was found to be 340 nm and the calibration curve was prepared for a range of 10-50 µg/mL. The regression equation was obtained to be y=0.013x-0.013 with R2 value of 0.998 (Figure 1).

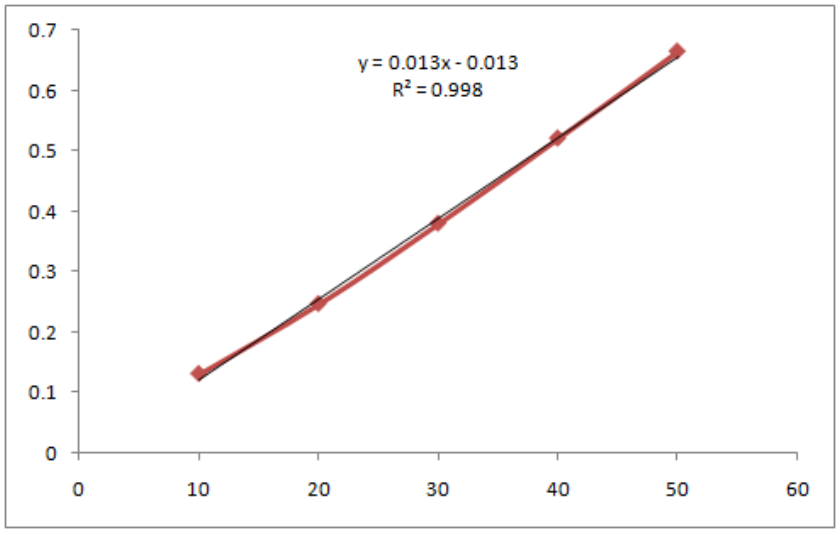

Figure 1: Standard curve of Metronidazole.

***In vitro* digestion of chitosan by probiotics**

The *in vitro* digestion study of chitosan in presence of probiotic was carried out to assess the effect of intestinal microbial flora on chitosan. The *in vitro* digestion study of chitosan was performed in presence of the probiotic to evaluate the effect of the bacteria have on chitosan. The microbial flora of intestine has been known to degrade chitosan leading to a decrease in its viscosity. The reduction in viscosity of chitosan was witnessed at the 24 h.

Flow properties of the granular blends

The angle of repose was found to be in the range of 25°71' to 27°41'. The bulk density value ranged from 0.42 to 0.46 g/cm^3 while the tapped density value ranged from 0.48 to 0.52 g/cm^3. The granules prepared in the present work were found to have the Carr's Index between 15.68 to 11.53 % and the Hausner's ratio was found to be ranging from 1.186 to 1.130 (Table 3).

Table 3: Flow properties of the formulation blends.

Formulation	Angle of repose	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Index
F1	25°71'	0.43	0.51	1.186	15.68
F2	27°37'	0.42	0.48	1.142	12.5
F3	27°41'	0.44	0.51	1.159	13.72
F4	26°38'	0.46	0.52	1.130	11.53
F5	27°10'	0.44	0.51	1.159	13.72

Evaluation of metronidazole colon targeting formulations

All the formulated matrix tablets were evaluated for weight variation, hardness, thickness, drug content and friability. The thickness of all the tablets was found to be 7.2-7.4 mm while the hardness was 6 kg/cm^2. The friability of all the formulations was less than 1% while the weight variation was 1.1 to 1.5 %. The drug content ranged from 97.9 to 98.7 % in the formulations (Table 4).

Table 4: Characteristics of the tablet formulations.

Batch Code	Thickness (mm)	Hardness (Kg/cm^2)	Weight variation (%)	Friability (%)	Drug Content (%)
F1	7.2	6	1.4	0.8	97.9
F2	7.3	6	1.5	0.8	98.2
F3	7.4	6	1.1	0.7	98.7
F4	7.2	6	1.2	0.8	98.1
F5	7.4	6	1.3	0.8	98.4

***In vitro* drug release study**

The tablet formulations were investigated for the *in vitro* drug release in absence and presence of rat caecal content and it was found that the release of drug from the tablets was affected by the presence of probiotic. The rat caecal content was able to increase the drug release from all the formulation (Figure 2 and 3).

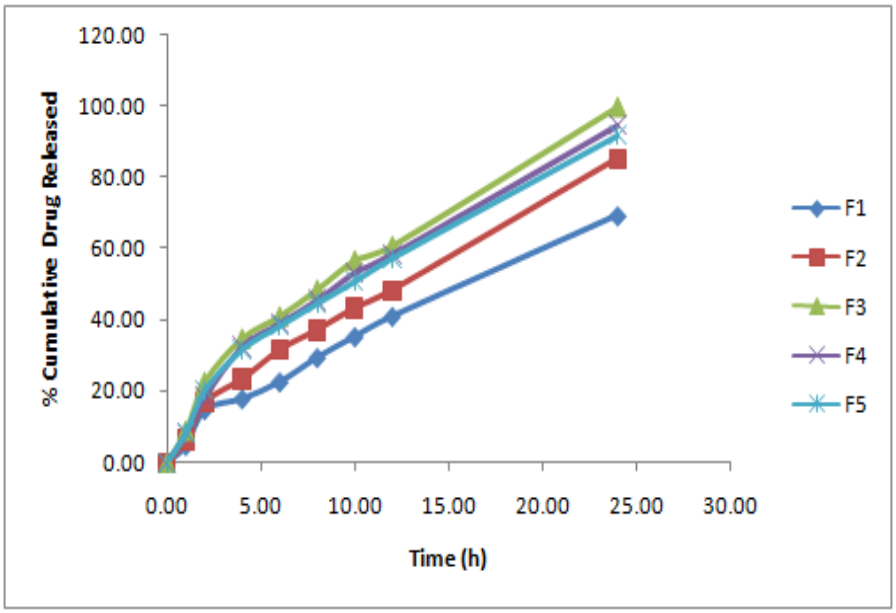

Figure 2: Percent release of metronidazole in presence of rat caecal content.

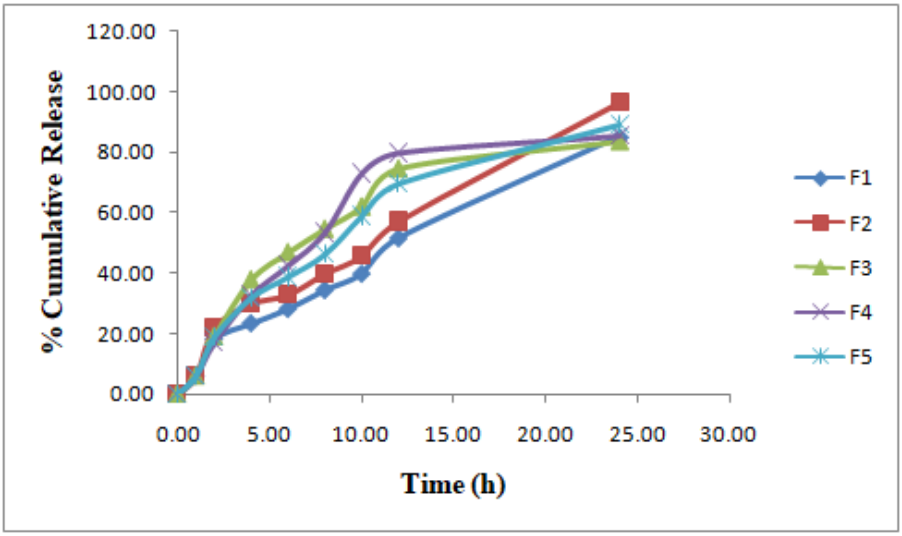

Figure 3: Percent release of metronidazole in absence of rat caecal content.

The *in vitro* release was assessed in absence as well as presence of rat caecal content. In absence of the caecal medium, the release of drug from formulation containing 20% chitosan (F1) was only 69.31% in 24 h whereas the other formulations released more than 85%. It was also observed that increasing the concentration of probiotic was able to increase the drug release from the formulations suggesting the assistive role of the probiotic in degradation of chitosan in the colonic microflora.

CONCLUSION

In the present study, colon specific matrix tabletes loaded with metronidazole were prepared using wet granulation method employing chitosan as the polymer and Rhamno G as the probiotic to aid colon targeting. The results obtained showed that this methodology was able to produce colonic release of the majority of drug even in the absence of colonic microflora and also produced sustained release of drug from the formulations.

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