FORMULATION AND GAMMA SCINTIGRAPHIC EVALUATION OF COLON SPECIFIC TINIDAZOLE TABLETS IN HEALTHY HUMAN VOLUNTEERS

Bhawna Gauri*[1,3] Dimple Chopra[1], Shailendra K Singh[2], Lovekesh Nagpal[3]

***[1] Division of Pharmaceutics, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala (Punjab)-India**

***[2] Division of Drug Delivery and Research, Department of Pharmaceutical Sciences, Guru Jambeshwar University of Science and Technology, Hisar-125001, (Haryana)-India**

***[3] Division of Pharmaceutics, Department of Pharmaceutical Sciences, Lord Shiva College of Pharmacy, Affiliated to Pt. B. D. Sharma University of Health Sciences, Rohtak-124001, (Haryana) India**

ABSTRACT

The objective of our study was to develop single-unit colon targeted drug delivery system of tinidazole using pH dependent methacrylic acid polymers and/or time dependent polymeric combination, which slowly release the drug to colon. Lactose-based tinidazole placebo tablets were coated using various ratios of two co-polymers, Eudragits®L100 and Eudragits®S100 (weight by weight) (w/w) 4:0, 3:1, 2:2, 1:3 and 0:4 respectively by coating method and Ethylcellulose and shellac 5:0, 2.5:2.5, 1:4, 0:5. The tablets were evaluated for various in process quality control parameters, in-vitro drug release studies and in-vivo gamma scintigraphic studies. The in-vitro drug dissolution data obtained from coated tablets demonstrated that dissolution rate of the coated tablets was dependent on (i) polymer combination ratio (ii) pH of media (iii) coating levels of the tablets. The transit profiles in two healthy volunteers by gamma scintigraphy demonstrated that the tablets were able to pass through the stomach and small intestine intact and could safely reach the distal end of the small intestine, where the system began to release the drug contained in the core tablet. For both of the volunteers, disintegration of the tablets occurred in the ascending colon, which had highlighted the potential of this system for colonic drug delivery. The result also demonstrated that the formulation can be adjusted to deliver the drug at any target site of the intestinal region of the gastrointestinal tract on the basis of pH variability. So, it concluded that colon targeted drug delivery system prepared herein, by means of regular coating techniques, were able to achieve site-specific drug delivery targeting to the colon following oral administration and provide a promising strategy to control drug release targeting the desired lower gastrointestinal region.

Keywords: Colon targeting, tinidazole, Gamma Scintigraphy, pH dependent system, time dependent system, In vivo study

INTRODUCTION

Colon drug delivery has raised significant importance in the field of pharmacotherapy during last few years. Diseases like inflammatory bowel disease (IBD) are treated effectively by delivering drugs to colon through targeting (1,2). Other colonic disorders like amoebiasis, crohn's disease, ulcerative colitis and colorectal carcinoma are also treated effectively by delivering active moiety locally. These systems are usually coated systems which minimize release/absorption of drug in stomach and small intestine but deliver drugs to the colon (3,4). Colonic drug delivery systems (CDDS) would be advantageous when a delay in absorption is desirable from a therapeutically point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis (5).

Since, amoebiasis is an infection of large intestine caused by protozoan parasite *Entamoeba histolytica*. Tinidazole and metronidazole are drugs of choice in the treatment of the amoebiasis, anaerobic infections and are to be delivered to the colon for their effective action against trophozoites of *E. histolytica* that reside in lumen of the caecum, large intestine (6). Tinidazole is used at dose of 2.0 gm/day orally with food for 3 days. As the conventional tablets are absorbed from the stomach, side effect like nausea, vomiting, metallic

Corresponding author:
Email: bhawna.gauri@gmail.com

taste and headache are observed. Therefore targeting the drugs specifically to the colon is advantageous in treatment of diseases such as amoebiasis, crohn's disease, ulcerative colitis and colorectal cancer (7). Colonic systems has lot of challenges like minimizing systematic degradation of drug in upper GI tract, protection of protein and peptide drugs from hydrolysis and enzymatic degradation in duodenum and jejunum etc. A carefully designed colon therapeutic system could achieve maximum drug release in colon and elicit high activity with minimum systemic adverse effects. The specific drug release in colon provides a sufficient time interval between administration and onset of action which proves to be useful in treating chronic diseases such as asthma and arthritis (8). The colon is believed to be a suitable site for absorption of drugs due to following reasons: (i) Less diversity and intensity of digestive enzymes. (ii) Comparatively proteolytic activity of colon mucosa is much less than that observed in the small intestine, thus controlled drug delivery system protects peptide drugs from hydrolysis and enzymatic degradation in the duodenum and jejunum and eventually releases drugs in the ileum or colon which leads to greater systemic bioavailability. (iii) Colon is rich in lymphoid tissue, uptake of antigens into the mast cells of the colonic mucosa produces rapid local production of antibodies and this helps in efficient vaccine delivery. (iv) Colon has a long residence time (up to 5 days) and is highly responsive to absorption enhancers (9). Some strategies currently available for colon targeting are prodrug formulations (10, 11), use of coating techniques to pH sensitive polymers (12, 9), timed release systems (4), osmotic systems (13, 14), pressure controlled drug delivery systems (15) and by utilizing colon specific biodegradable polymers (16). Among the above mentioned strategies, coated systems seems to be superior in preventing premature drug release in stomach and small intestine, while beginning to release the active agents at the proximal colon and utilizing the advantage of luminal pH in the ileum and/or microbial enzymes in the colon. The pH dependent systems exploit the generally accepted view that pH of the human gastrointestinal (GI) tract increases progressively from the stomach (pH 2-3), small intestine (pH 6.5-7.0) to the colon (pH 7.0-8.0). Most commonly used pH-dependent coating polymers are methacrylic acid co-polymers Eudragits®L100, Eudragits®S100 and Eudragits®L100-55 which dissolves at pH 6.0, 7.0 and 5.5 respectively (1). A large number of polysaccharides such as pectin, amylose, guar gum, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans, dextrin and locust bean gum have been investigated for their use in colon targeted drug delivery systems in other systems. Hence, none of these polymers are suitable to be used alone for coating of dosage forms that would start releasing the drug at pH 6.5 although this has been generally accepted as the desired pH for colon targeted drug delivery. Taking advantage of the highest pH value of colonic content, the dosage form containing the active drug in a core is coated with combinations of pH dependent methacrylic acid polymers or time dependent polymers, which dissolves in the colon and lot of benefits would be acquired in terms of improve in safety and reduction in toxicity when treating local or systemic chronic diseases.

Thus, in the present investigation we developed pH- and time-dependent released system for colon targeted oral drug delivery of the tinidazole that would allow the dosage forms to release the drug to the colon and to demonstrate its site-specificity in the colon for the treatment of amoebiasis. Besides, it was intended to exploit the typical pharmaceutical coating technology to attain colon-specific delivery.

MATERIALS AND METHODS

Materials

Tinidazole IP (98 to 101.2% pure) (M/s J. B Chemical & Pharmaceutical Ltd, Mumbai), Eudragit® L100, Eudragit® S100 was obtained as gift sample from Degussa India Pvt. Ltd, Mumbai. Ethylcellulose was obtained from Colorcon Co., China and Shellac was procured from local market. Lactose, Microcrystalline cellulose (MCC) (Avicel™ PH 102), Polyvinylpyrolidone (PVP) K-30 was procured from CDH Company, New Delhi and was of USP/NF quality. Other excipients were of standard pharmaceutical grade.

Methods

(A) Preparation of tinidazole coated tablets

The tinidazole core tablets were prepared employing wet granulation technique (6, 18) using PVP K-30 as binder. The wet mass was passed through a mesh (1680µm) and the dried granules passed through a mesh (1190µm) and lubricated with a mixture of talc-magnesium stearate (2:1). The lubricated granules were first evaluated for flow properties (19) (angle of repose, tapped bulk density

(TBD), loose bulk density (LBD) and compressibility index (CI)) and then compressed using 8.0 mm size round, flat and plain punches on a single station tableting machine (M/s Cadmuch Machinery, India). Table 1 shows the composition of a typical tinidazole

Table 1: Composition of tinidazole colon specific core tablet

Ingredients	Quantity/tablet
Tinidazole	200 mg
Lactose	150 mg
Microcrystalline cellulose	34 mg
Polyvinylpyrrolidone-K30	q. s.
Magnesium stearate	8 mg
Talc	8 mg
Total Average Weight	400 mg

core tablet. The core tablets were evaluated for hardness, content uniformity, friability and disintegration. After meeting with standards, the core tablets were then subjected to coating. A polymeric spray coating dispersion was prepared using mixture of Eudragit® L-100 and Eudragit® S-100 in different ratio as depicted in table 2. Apart from this, Shellac and Ethylcellulose were also used in combination as coating polymer for preparation of coating dispersion for tablets as depicted in 3 (18). The enteric coating solution was prepared by dissolving Ethylcellulose and Shellac in ethanolic and acetone mixture (20). Coating dispersion formulations also contains polyethylene glycol as plasticizer, ethanol and acetone as organic solvent in it and talc as glidant. The placebo core tablets were coated up to 10-12%w/w (of total solid applied) in conventional laboratory coating pan fitted with three baffles placed at angle of 120°.

(B) Evaluation of Tablets:

(i) Evaluation of Granules

Tinidazole granules prepared above for core tablets was evaluated for their angle of repose (^{0}), Loose bulk density (LBD), Tapped bulk density (TBD) by using USP method II (21) and Compressibility Index (CI). LBD (9) and TBD (9) were determined using formula: LBD = weight of powder/volume of packing, TBD = weight of powder/tapped volume of the packing. CI (9) of the granules was determined by using formula. CI (%) = [(TBD-LBD)/TBD]*100

(ii) Physical characteristics of fabricated tablets

The weight variation test was conducted as per specifications. To study the weight variation test 20 tablets from each batch were taken and weighted using an electronic balance (AW-220, Shimadzu) and test was performed according to the official method (21) from average weight of prepared tablets, individual deviations was determined. The crushing strength (18) (Kg/cm^2) of prepared tablets of tinidazole was determined by using Monsanto tablet hardness tester (Cadmach Pvt. Ltd) by using 6 tablets

Table 2: Coating dispersion formulation for Eudragits

Batch Code	Tinidazole core weight	Eudragit ®L100	Eudragit®S100	Ethanol	Acetone	PEG-400
MLS1	400 mg	4.0 gm	0.0 gm	24.0 ml	25.0 ml	1.0 ml
MLS2	400 mg	3.0 gm	1,0 gm	24.0 ml	25.0 ml	1.0 ml
MLS3	400 mg	2.0 gm	2.0 gm	24.0 ml	25.0 ml	1.0 ml
MLS4	400 mg	1.0 gm	3.0 gm	24.0 ml	25.0 ml	1.0 ml
MLS5	400 mg	0.0 gm	4.0 gm	24.0 ml	25.0 ml	1.0 ml
MLS6	400 mg	Plain core tinidazole tablets without coating				

Table 3: Coating dispersion formula for Ethyl cellulose and shellac.

Batch Code	Tinidazole core tablet weight	Ethyl cellulose (in gm)	Shellac (in gm)	Dicholoromethane (in ml)	Ethanol (in mL)	Acetone (in ml)	PEG-400
T1	400 mg	5.0	-	5.0	4.0	40	1.0
T2	400 mg	2.5	2.5	5.0	4.0	40	1.0
T3	400 mg	1.0	4.0	5.0	4.0	40	1.0
T4	400 mg	-	5.0	5.0	4.0	40	1.0

of each batch formulation. Friability test of tablets was determined by Roche Friabilator (Electrolab, Mumbai, India) at 25 rpm for 4 minutes with 20 tablets dropping from height of 6 inches with each revolution (21).

Friability= (Initial Weight-Final Weight)/Initial weight *100

Thickness of compression coated tablets was measured using Digital Micrometer (Digital Caliper, Aerospace, India).

(iii) *In vitro* drug dissolution studies

Preparation of 4% rat cecal content medium

Male albino rats weighing 105-115 gm were used for the study. The care of the rats was in accordance with the institutional guidelines. It has been reported that rat caecal content medium at 4% w/v level obtained after 7 days of enzymatic induction with 1 mL of 2% w/w guar gum dispersion provide the best conditions for assessing the susceptibility of guar gum to colonic bacterial degradation (22). Hence, the rat caecal content medium was prepared as described previously (22). The rats were treated with guar gum dispersion for inducing the enzymes specifically acting on guar gum. The procedure involved oral treatment of rats with 1 mL of 2% w/v guar gum dispersion for 7 days. Thirty minutes before the commencement of drug release studies, six rats were sacrificed and caecai were traced, ligated at the both ends with thread, dissected and immediately transferred into buffer saline solution pH 6.8 to obtain 4% w/v concentration caecal content. Phosphate buffer saline (pH 6.8) was previously bubbled with carbon dioxide gas to maintain an anaerobic environment. As the caecum is naturally anaerobic, all the operations were carried out under carbon dioxide to maintain the anaerobic environment.

***In-vitro* drug dissolution studies**

Dissolution studies were carried out for all the formulations i.e. in triplicate, employing USP XXIII basket method (Apparatus 1) (Electrolab, Mumbai, India) using 900 mL 0.1 N HCL as the dissolution medium at 100 rpm and 37±0.5°C for 2 h as the average gastric emptying time is about 2 h. Then the dissolution medium was replaced with pH 7.4 phosphate buffer saline (900 mL) and dissolution studies were continued for another 3 h. The ability of formulated tablets of tinidazole to release the drug in physiological environment of colon was assessed by continuing the drug dissolution studies in pH 6.8 phosphate buffer saline (900 mL) for another 15 h. An aliquot of sample was periodically withdrawn at suitable intervals and volume was replaced with equivalent amount of respective dissolution medium. The samples were filtered and analyzed after suitable dilution at 278 nm, 318 nm and 320 nm for 0.1 N HCL, pH 6.8 phosphate buffer and pH 7.4 phosphate buffers respectively using UV-VIS spectrophotometer (Shimadzu 1601, Japan).

***In-vitro* drug dissolution studies in presence of rat caecal contents**

Dissolution studies were carried out for all the formulations in triplicate, employing USP XXIII basket method (Apparatus 1) (Electrolab, Mumbai, India) using 900 mL simulated gastric fluid (SGF) (pH 1.2) as the dissolution medium at 100 rpm and 37±0.5°C for 2 h as the average gastric emptying time is about 2 h. Then the dissolution medium was replaced with

Table 4: Evaluation parameters for granules of various formulations

Name of Batch	LBD* (g/ml)	TBD* (g/ml)	Compressibility Index* (%)	Angle of Repose* (°)
Tinidazole core tablet granules	0.592±0.01	0.719±0.03	17.6±0.02	25.23±0.01

*Mean ± SD, n =3

900 mL of simulated small intestinal fluid (SSIF) (pH 7.4) and dissolution studies were continued for another 3 h. The ability of formulated tablets of tinidazole to release the drug in physiological environment of colon was assessed by continuing the drug release in presence of rat caecal contents with slight modification. The swollen tinidazole tablets obtain after completing the dissolution study in SGF (2 h) and SSIF (3 h) were placed in the baskets of the dissolution apparatus and immersed in pH 6.8 phosphate buffer containing rat caecal content with slight modification. A beaker (capacity 250 mL) containing 100 mL of rat caecal content medium was immersed in the water maintained in the 1000 mL vessel, which, in turn, was in the water bath of the apparatus. As the caecum is naturally anaerobic, the experiment was carried out with continuous CO_2 supply into the beakers. At various time intervals, 5 mL of the dissolution sample was withdrawn without a pre-filter and replaced with 5 mL of fresh simulated colonic fluid (SCF) (pH 6.8) bubbled with CO_2, and the experiment was continued for another 15 h as the usual colonic transit time is 20-22 h. To the samples, 2 mL of methanol was added to ensure solubility of finely suspended drug particles released due to break down of the coat by the caecal enzymes. The volume was made up to 10 mL with SCF (pH 6.8), centrifuged and the supernatant was filtered through a bacteria-proof filter and the filtrate was analyzed for tinidazole content at 320nm using UV-VIS spectrophotometer (Shimadzu 1601, Japan).

Table 5: Physical characterization of pH dependent colon specific tinidazole coated tablets

Formulation code	Thickness* (mm)	Friability* (%)	Weight variation *(mg)	Hardness* (Kg/cm^2)
MLS1	3.41 ± 0.05	0.16 ± 0.1	449.79 ± 1.22	8.2±0.2
MLS2	3.32 ± 0.03	0.12 ± 0.08	446.55 ± 2.69	8.4±0.3
MLS3	3.12 ± 0.02	0.21 ± 0.05	447.13 ± 1.32	8.4±0.2
MLS4	3.38 ± 0.04	0.15±0.04	447.15 ± 2.99	8.2±0.3
MLS5	3.37 ± 0.03	0.12±0.08	448.32 ± 2.48	7.9±0.2
MLS6	3.02 ± 0.04	0.28±0.07	408.10 ± 2.10	6.8±0.2

*Mean ± SD, n =3

(iv) Statistical Analysis:

Data are presented as mean±standard deviation. Release profiles of various batches were compared using Analysis of variance test (ANOVA) which was performed to check whether there is significant difference among the different formulations and $p < 0.05$ was considered to be significant.

(v) Gamma-Scintigraphic Studies:

The efficacy of colon targeted tablets of tinidazole to the human colon was assessed by subjecting them to γ-scintigraphic studies in healthy human volunteers. The studies were conducted at Institute of nuclear medicine and allied sciences (INMAS), New Delhi (Registration no: AM/INM/UM/Msc) with all previously taking ethical consents. The best formulation was subjected to in vivo investigation in healthy human volunteers for the determination of its colon targeted ability. Tinidazole best formulation was radiolabelled by neutron activation. This technique involves the incorporation of small amount (1 mg) of isotropically enriched metastable isotope of technicium-99m into the formulation prior to manufacturer. After informing the volunteers about the protocol and requisite precautions required during the study, written consent forms were obtained from the volunteers. The volunteers were asked to take the light breakfast. One hour after breakfast, radioactive technetium-99m labeled tablet of tinidazole formulation (T2) was administered with 200mL of UV irradiated and filtered potable water. Following oral administration of the radio labeled tablets, static images (60 sec/image) were acquired under dual head gamma camera. The γ-imaging was done at different time intervals of 0, 1, 2, 3, 4, 5, 6, and 7h using gamma camera (General Electrix Maxicamera). A final image was taken after 24 hour of post dose to check final fate of dosage form. The images were recorded using a computer system and stored on optical disk for subsequent analysis.

Table 6: Physical characterization of time dependent colon specific tinidazole coated tablets

Formulation code	Thickness* (mm)	Friability* (%)	Weight variation *(mg)	Hardness* (Kg/cm^2)
T1	3.31±0.04	0.12±0.2	442.69±2.22	8.8±0.4
T2	3.42±0.05	0.13±0.06	443.85±2.59	8.7±0.5
T3	3.22±0.06	0.17±0.07	445.36±1.28	8.8±0.5
T4	3.67±0.08	0.13±0.05	451.45±1.95	8.6±0.6

*Mean ± SD, n =3

RESULTS AND DISCUSSION

The present study is aimed at development of single-unit oral colon targeted formulations of tinidazole using different polymers and to identify the most suitable polymeric combinations among chosen polymers which slowly release the drug at target site, to minimize the gastric side effects and increased patient compliance. Various coated formulations were prepared and details along with the formulation codes are given in Table 1. Ideally, the drug delivery system targeted to colon should remain intact in the stomach and small intestine, thereby releasing the drug in the colon. Hence, attempts were made to formulate the tablets using Eudragit L100 and Eudragit S100 combination and Ethyl cellulose and pectin combination of polymers.

(i) Evaluation of granules and tinidazole tablets:

For this, granules of different formulations were evaluated for angle of repose, LBD, TBD and CI. Granules indicated fair to good flowability with angle ranges from 20-25°. The results of LBD, TBD and CI are mentioned in table-4. All the tablet formulations showed acceptable properties and complied with the in house specifications for weight variation, thickness, drug content, hardness and friability were within acceptable official limits as specified in table 5.

(ii) In vitro dissolution studies:

pH dependent tinidazole coated tablets:

Tinidazole core tablets were coated with combination of Eudragit®L100-Eudragit®S100 methacrylic acid pH-sensitive copolymers, and their release characteristics were examined by three sequential dissolution media with different pH values in order to mimic pH changes along GI tract. We investigated the effect of combination ratios of Eudragit® L100-Eudragit® S100 in the coating

formulation on the release profiles of tinidazole polymeric combination) with rat cecal content and

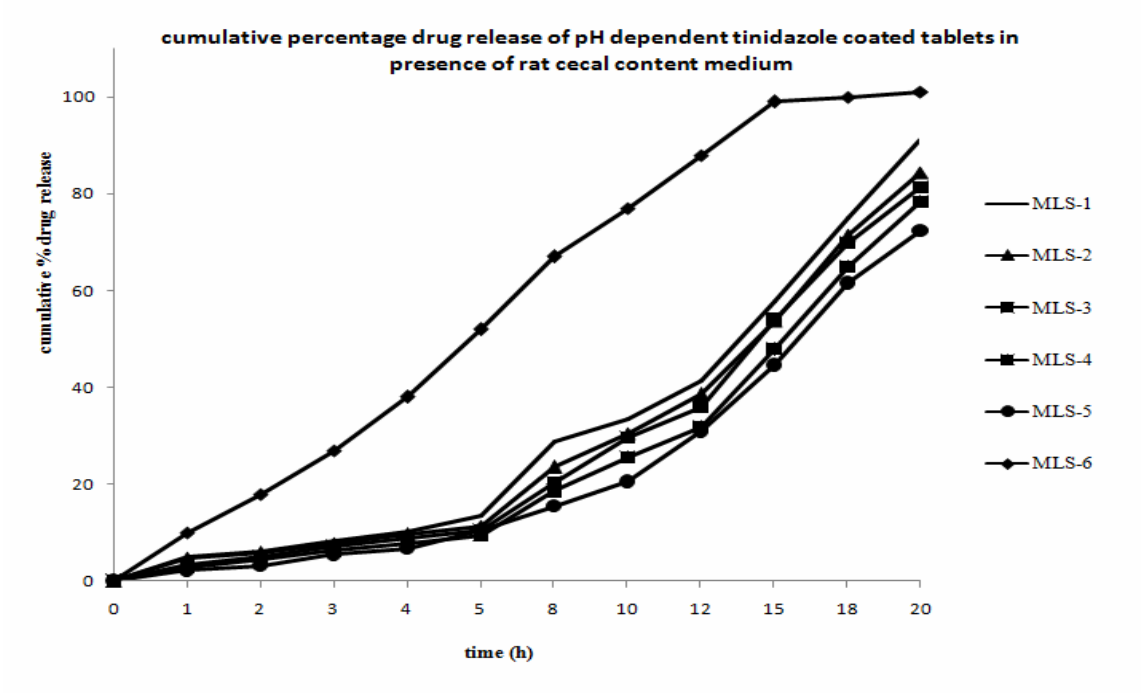

Figure 1: Dissolution profiles of pH dependent tinidazole coated tablets in changing pH media and simulated colon fluid. Drug release study was also conducted in PBS from 5th -20th hours for coated formulation in order to understand the influence of colonic fluid on the drug release profile.

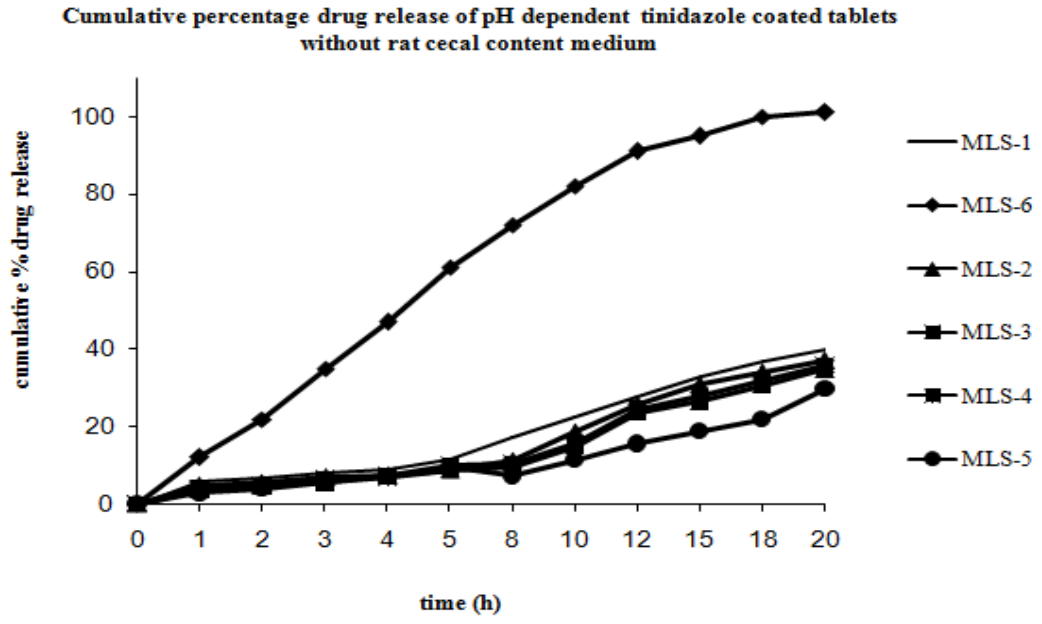

Figure 2: Dissolution profiles of pH dependent tinidazole coated tablets without rat cecal medium

coated tablets. It indicates dissolution profiles of coated formulations (pH sensitive eudragits without rat cecal contents respectively, as shown in figure 1 and 2. As it reveals that there is a direct

relationship between the decrease in the dissolution rates and with increase in the Eudragit®S100 content in the formulations. All the coated formulations of tinidazole tablets coated at 10-12% levels met the USP criteria for the enteric performance test in SGF (for 0-2nd hr), SSIF (2nd -5th hr) and SCF (5th -20th hr) except uncoated tablets (MLS-6). The dissolution profiles of all tested formulations are indicates that formulation MLS-6 was disintegrated prematurely within 2 hr, releasing the majority of drug (more than 60%) in the simulated gastric fluid (SGF) and simulated intestinal fluid (SSIF) and fails to control the colon targeting. This is attributed to absence of polymeric coating and resulted in the disintegration/erosion of the tablet at early stage. This also accounts for a fast release of the drug from the tablet. From the data it was found that delayed release pattern could be observed with eudragit L-100, if more than 25% of eudragit L-100 was used. However the formulation would not be cost effective. As expected, the tablets coated only with Eudragit®S100 (0:4, MLS-5) has the slowest dissolution rate and started release of the drug with in 30 min and only 20.6% drug was released in 10 hrs. Combination formulations (i.e., formulation MLS-2, MLS-3, MLS-4,) released 25-40% tinidazole. The slower release profiles were observed with MLS-5, MLS-2, MLS-3 whereas the maximum drug release was observed with MLS-6. The drug release profile showed that, MLS-6 releases 80% of drug whereas MLS-1 released about 34% of drug in 10 hrs. The percentage of drug release vs. time plot shows that the dissolution rate was inversely proportional to the thickness of coat applied. The dissolution profiles of tinidazole obtained from tested formulations clearly demonstrate that a significant difference was observed in percentage drug released for different coating concentrations. The results show that the release of drug can be manipulated by changing the Eudragits®L100 & Eudragits®S100 ratio in the combination in the pH range of 6.0 and 7.0. The increased disintegration/dissolution time was obtained from almost all the formulations under all testing conditions with the higher levels of coating of those with lower levels, demonstrating the effect of coating thickness on disintegration/dissolution rate. It was expected that the longer time will be taken to solubilize the thicker film.

Time dependent tinidazole coated tablets:

On the basis of transit time of prepared formulations in colon, we designed time-dependent colon-specific drug delivery system to achieve no substantial drug release in the upper part of gastrointestinal tract and a significant drug release after arrival at colon. Tinidazole formulations were coated with water insoluble ethylcellulose and shellac combination along with PEG-400 as channeling agent by means of coating technology. Generally, the coating layer of EC and shellac was mainly hydrophobic and moderately impermeable to

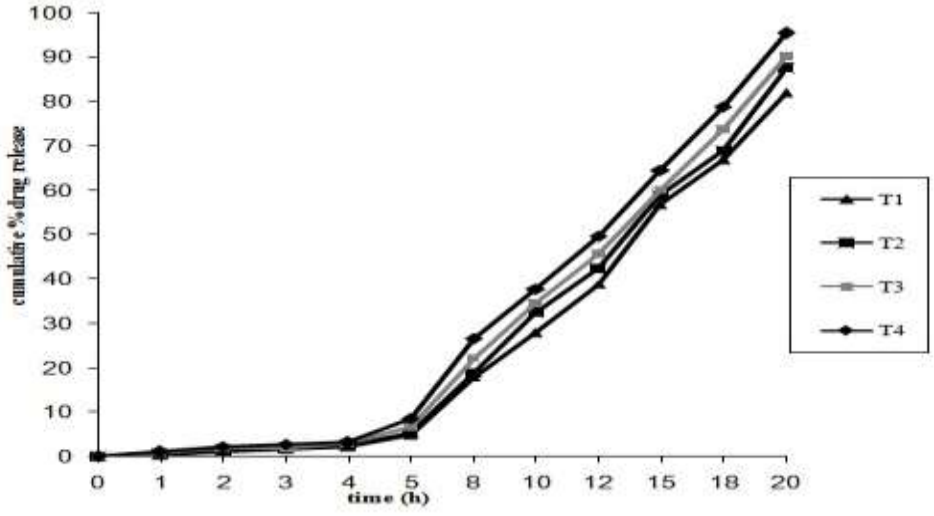

Figure-3: Dissolution profiles of time dependent tinidazole coated tablets in changing pH media and simulated colon fluid. Drug release study was also conducted in PBS from 5th -20th hours for coated formulation in order to understand the influence of colonic fluid on the drug release profile.

water molecules. The water-soluble channeling agent was rapidly dissolved in water/body fluids, resulting in the formation of numerous micro pores across the coating layer and helps in penetration of water molecules into the tablet core to cross the hydrophobic coating. Water uptake by these channels caused the expansion of the disintegrating agent within the tablet core, resulting in a gradually pressure built up in the coated tablets. Once the inside pressure exceeded the critical disrupted value that the coating layer would be ruptured and release drug in burst manner, thus forming the delay effect of drug release from the coated tablets. Taken together, the formation of micro-pores was the first crucial step, and the critical disrupted pressure exerted by the coating layers were the key factors for time-dependent controlled release (4). Hence, initially, no drug release was observed in acidic pH till 2hr and less than 10% drug release in phosphate buffer pH 7.4 till 5hr in presence of rat cecal content (Figure-3). As it was indicated from figure 3 that maximum drug release was observed with T4 formulation and minimum release was indicated in T1 formulation. It was observed that there is direct relationship between concentration of Ethylcellulose and rate of drug dissolutions. As the concentration of Ethylcellulose in coat solution increases it leads to decrease in dissolution rate. Drug release studies were also enhanced in presence of rat cecal content when compared without rat cecal content as indicated in figure-3 & figure-4 respectively. The high level of ethylcellulose in the coat however, was successful in controlling the percentage release of drug. The characteristics of Shellac and Ethylcellulose hence it depends on the ratio and coating concentration of Shellac and Ethylcellulose used in the coat and colon. It is well known that the addition of lactose can improve the flow and bond properties of other excipients. In particular, lactose with higher water solubility might also facilitate the disintegration and dissolution of solid dosage forms. Thus, lactose was mixed with other excipients, which not only improved the flow ability but also to monitored the lag time of the tablets. It is also evidenced that pH values of media did not exert a significant effect on the tinidazole release pattern from the coated tablets. This was reasonably expected, since the channeling agent PEG 400 was pH-insensitive and formation of the micro-pores was not affected by pH.

(iii) Selection of Best Formulation:

The best formulations (T2) were selected based on the percentage of drug release in the targeted time period, with an intent to have <10% release at the end of 5 h (which is considered as lag time for the system to reach colon) and 100% release within 1 after the system reaches the colon. Finally, the selected formulations were allowed for gamma scintigraphic studies.

(iv) Scintigraphic Data Analysis:

The data were analyzed to provide information on parameters evaluated for the tablets were time of gastric emptying, small intestinal transit time, colon arrival, initial disintegration, and complete disintegration. Gastric emptying time is defined as the time midway between the image where the

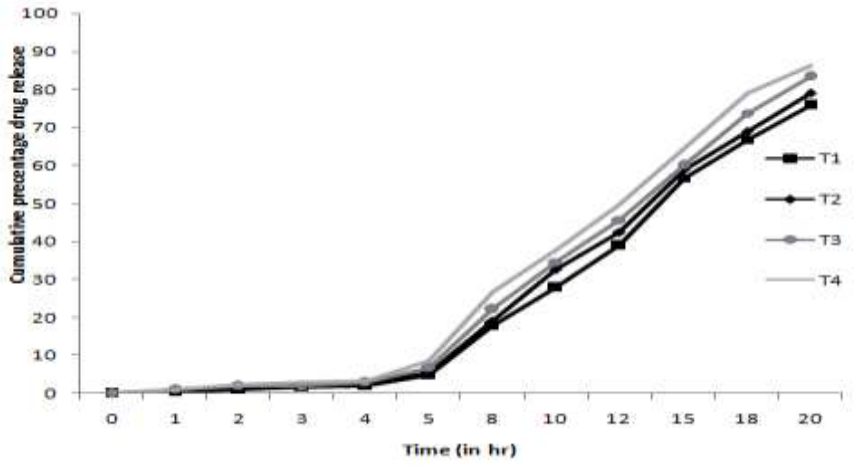

Figure-4: Dissolution profiles of time dependent tinidazole coated tablets without rat cecal contents.

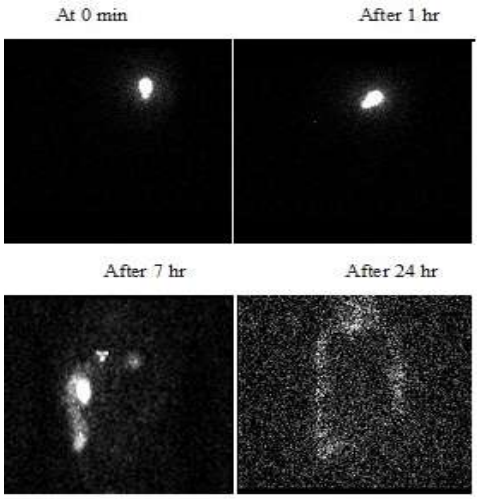

Figure 5: Static scintigraphic images of Volunteer-1 following oral administration of 99mTc-labelled selected formulation (T2) at different time intervals.

tablet core was last seen in the stomach, and the image where the tablet core was first observed in the small intestine, with similar parameters applied to colon arrival time. Where the tablet completely disintegrated in the stomach, gastric emptying was recorded as the time of onset and completes emptying of the disintegrated tablet. Small intestinal transit was defined as the time period between tablet core gastric emptying time and colon arrival for the tablet formulations. Mean total time for tablet disintegration was determined by subtracting the initial disintegration from the complete disintegration time for each animal. Initial tablet disintegration was defined as the time point midway between the last image where no tablet disintegration was observed, and the first image where evidence of disintegration of the tablet core was observed in two or more consecutive images. Complete tablet disintegration was determined as the time point at which the presence of a distinct tablet core could no longer be detected. Transit data of tinidazole formulation (T2) are given in figure-5 & -6 in healthy human volunteer using Tc-99m as a tracer. From the scintigraphs (Figure-5, & -6) taken at regular time intervals, the gastric emptying time was found to be 2-3 h and the small intestinal transit time was 3-5 h. The colonic arrival time of the tablets was 5-6 h in both volunteers. The tracer was not released either in stomach or in small intestine of both the volunteers. Although formulation showed subtle sign of initial tablet disintegration in the small intestine in all the subjects, and all tablets remained essentially intact during their transit through the small bowel with only a very small amount of disintegration occurring through before formulation reached to the colon. On entering the ascending colon only, the tablets began to release the tracer indicating the dissolution of ethylcellulose and shellac coats. Scintigraphs (Figure-5) showing intact tablet in the small intestine (3h), the commencement of release of the drug with tracer (5h), uniform distribution of the released tracer across the entire colon (24h) in volunteer 1. At the end of 24 h, the released tracer was found distributed in different segments of the colon in both volunteers. However, in volunteer 2 (Figure-6), the released tracer was concentrated in ascending colon even after 7h of tablet administration. In the present study, only anterior images of 60 seconds duration were taken by placing the volunteers in supine position using a gamma-camera. Hence, no data with respect to percent tracer released in different segments of colon could be generated. The in-vivo studies clearly demonstrated that the developed system could deliver the drug to the colon successfully.

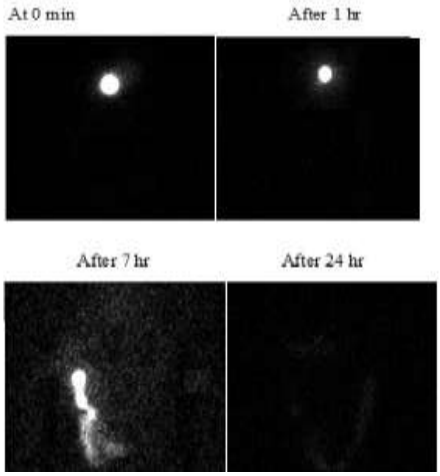

Figure 6: Scintigraphic images of Volunteer-2 following oral administration of 99mTc-labelled selected formulation (T2) at different time intervals.

CONCLUSION

Successful colonic delivery requires careful consideration of a number of factors, including the properties of the drug, the type of delivery system

and its interaction with the healthy or diseased gut. Although a number of formulations have been proposed as colonic delivery vehicles, most lack the necessary of site specificity. The only universal system currently marketed is time dependent systems (T2 formulation), and all other systems has shown pre-release of the drug in GI tract. All the physicochemical studies performed on drug, granules and the formulations were falling within the limit and shown feasibility in their formulations. From the result obtained on drug release study of the spray coated formulations found to be suitable for colon-specific drug delivery. The time dependent tablets showed a slightly slower rate of drug release and slightly high residence time when compared with pH dependent coated tablets. In term of release kinetic studies, the best formulations i.e., T2 showed the desired zero order drug release. Hence, this special drug delivery system is needed to minimize the hazardous effects and to increase the effectiveness of the drug and there is a need of to investigate a number of indigenously available retardant materials to make the concept of colon specific drug delivery more viable for the industry at more economical way.

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