Development of time and pH dependent controlled release colon specific delivery of tinidazole

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ABSTRACT

Purpose: Tinidazole is used in treatment of amoebiasis and other protozoal infections in doses of 2.0 g/ day (60 mg/kg) for three days. In the present paper, controlled release formulation of tinidazole was developed with an objective to achieve colon specific drug delivery with reduced frequency of dosing, to minimize gastric side effects and thus to increase patient compliance.

Methods: Matrix systems of tinidazole (500 mg) were prepared by using swellable and pH dependent polymers like hydroxypropyl methylcellulose (HPMC K4M and K15M) and eudragit (eudragit L-100 and S-100). Prepared tablets were enteric coated in order to overcome variability in gastric emptying time and delay in the release, to reduce gastric side effects and to provide prolonged localized action in colon. Process of manufacture was optimized during the scale up studies. Bioavailability study (using parallel group design) was carried of on conventional marketed, developed uncoated and enteric coated tablets in healthy human volunteers.

Results: Bioavailability study showed that greater portion of tinidazole was released in the large intestine and drug level in plasma was above 4 μg/mL in blood for 24 hours.

Conclusion: From the results of this study it appears that, the proposed single enteric coated tinidazole (500 mg) tablet per day could be used in place of 3-4 doses of 500 mg tinidazole conventional tablet with better control of drug release for targeted drug delivery. In addition developed colon-specific drug delivery system (CDDS) was relatively inexpensive and easy to manufacture using conventional pharmaceutical coating technique.

Keywords: Tinidazole, time and pH dependent controlled release, colon specific, eudragit, hydroxypropyl methyl cellulose

INTRODUCTION

Amoebiasis is an infection of large intestine caused by protozoan parasite, *Entamoeba histolytica*. Tinidazole and metronidazole are drugs of choice in the treatment of amoebiasis, also effective against anaerobic microorganisms 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 and adhere to colonic mucus and epithelial layers (1). Tinidazole is used at doses of 2.0 g/ day (60 mg/kg) for three days (2-4). From conventional formulation, water-soluble tinidazole is absorbed completely and rapidly through an enterohepatic circulation and acts on trophozoites present in large intestine (5-6). For this process, higher dose of about 2.0 g of tinidazole is required to achieve 4 μg/mL concentration of the drug in the plasma. As conventional tablets are absorbed from the stomach, side effects like nausea, metallic taste, vomiting and headache are observed. Therefore targeting the drug specifically to the colon is advantageous in treatment of diseases such as amoebiasis, crohn's disease, ulcerative colitis and colorectal cancer (7).

Among modified release oral dosage forms, increasing interest has currently turned to the design of systems to achieve time-specific and site-specific delivery of drugs. In the last decade, numerous clinical and animals studies have provided convincing evidence that pharmacokinetics and/or drug's effects-side effects can be modified by the circadian time and/or timing of the drug application within 24 hrs of a day. On the other hand, colon-specific drug delivery systems (CDDS) are developing as one

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of the site-specific drug delivery systems. Along with many applications in local and systemic delivery of drugs, CDDS would also be advantageous when a delay in absorption is desirable from therapeutic point of view (8-9).

Current delivery system, by means of combination of one or more controlled release mechanisms, hardly release drug in the upper part of gastrointestinal (GI) tract, but rapidly release drug in the colon following oral administration. In the case of CDDS specific drug delivery to the colon, lot of benefits would be acquired in terms of improve in safety and reduction in toxicity when treating local or systemic chronic diseases. First, for treatment of localized colonic diseases, the optimal drug delivery system, such as CDDS, should selectively deliver drug to the colon, but not to upper GI tract. For this reason, the drug concentration must be significantly low in the upper GI tract, while considerably higher in the colon, resulting in alleviated GI side effects (10-11).

CDDS are designed based on one of the following mechanisms with varying degrees of success: 1. prodrugs, 2. pH-sensitive polymer coating, 3. time-controlled dissolution and 4. microflora-activated drug release. The pH dependent systems are designed to release the drug in particular pH of the GI. Colon has a lower pH value (pH 6.5) than that of the small intestine (pH 7.0–7.8). Based on the concept that formulation leaves the stomach and arrives at the ileocaecal junction in about 6 hrs after administration as well as differences in pH throughout GIT, time and pH dependent pulsatile device for colonic targeting has been designed, for selective delivery of drug to colon. In brief, a typical CDDS configuration consists of a core tablet coated with a layer of polymer which contains the active ingredient along with one or more polysaccharides (e.g. lactulose) and other desirable excipients. During its transit through GI tract, the tablet remains intact in the stomach because of enteric protection, but enteric coating dissolves in small intestine, where the pH is above 6.

As a part of our continuing efforts towards development of novel drug delivery systems (12-14), herein we report development of time and pH dependent controlled release colon specific delivery system of tinidazole. Aim of this study was to explore the feasibility of time and pH dependent enteric-coated matrix system for treatment of amoebiasis with single daily dose of 500 mg tinidazole. Besides, it was intended to exploit the typical pharmaceutical coating technology to attain the time and pH dependent colon-specific drug delivery.

MATERIALS AND METHODS

Materials

Tinidazole IP (Aarti Drugs Ltd), eudragit S-100 and eudragit L-100 (Rohem Pharma), methocel K4M and K15M (Colorcon Asia Ltd), microcrystalline cellulose IP (NB enterprise), polyvinyl povidone K-30 (Povidone, USP/NF, Riyachem), cellulose acetate phthalate (Colorcon), magnesium stearate IP (Parag fine organic, Palghar), talc IP (ATOZ Traden) were obtained from Ajanta Pharma Ltd. as gift samples.

Standardization of drug and polymers

Tinidazole was standardized according to IP 1996 procedure for melting point, loss on drying, IR spectrum and assay (UV spectrophotometry and non-aqueous titration) (15). pH dependant polymers, eudragit L-100 and eudragit S-100, time dependant polymers HPMC K4M and HPMC K15M (according to USP XXIV), polyvinyl pyrolidone and cellulose acetate phthalate (CAP) (according to IP1996) were evaluated on the basis of the official monographs.

Preparation of tinidazole matrix tablets

Development of controlled release CDDS of tinidazole was attempted by designing many formulations based on the diffusion controlled matrix system. Two types of granulation techniques *viz.* intragranulation and extragranulation were investigated.

Intragranulation was used for eudragit polymers in which drug, polymer and MCC were granulated with isopropyl alcohol (IPA) while in the case of extragranulation, drug and MCC were granulated with IPA and then granules were mixed with polymer. For all batches non-aqueous wet granulation method was used (16).

In preparation of tablets using polymers eudragit L-100 and S-100, initially the drug, eudragit L-100/ S-100 and MCC were passed through sieve # 60 and this mixture was kneaded with IPA (formulae given in table 1). Since eudragit forms sticky plastic mass, IPA was carefully added and this mass was passed through sieve # 7. Granules were dried at 45 °C for 15-20 minutes then passed through sieve # 16 and mixed with talc and magnesium stearate. Tablet premix was compressed using 19.5 × 8.5 mm capsule shaped punches at hardness around 5-8 kg on single stroke tableting machine.

HPMC is a swellable polymer that shows time dependant release profile when the tablet comes into contact with liquids. HPMC K4M has viscosity of 3000-5600 m Pas and was used for matrix formulation. Batches TH1, TH2 and TH3 were prepared by intragranulation method as described in the method for eudragit. Binding with only HPMC K4M was not suitable for intragranulation. To strengthen granules, PVP K30 in IPA was used as binder. Batches TH4, TH5 and TH6 were prepared with same proportions as that of batches TH1, TH2 and TH3. Granules in this case were made by extragranulation method. Sieved drug and MCC were mixed and this mixture was kneaded with PVP solution (10% in IPA) for 7-9 min. This wet mass was passed through sieve # 7 and granules were dried at 60 °C for 15 minutes following drying in air for 10 minutes. Dry granules were passed through sieve # 36 and HPMC K4M was added accordingly in desired ratio. Talc and magnesium stearate were also mixed in graded proportion. Tablets of all HPMC K4M batches were compressed on six station rotary machine using 19.5 × 8.5 mm capsule shaped punches at hardness around 4.5 kg. HPMC K15M has higher viscosity than HPMC K4M. Formulations with HPMC K15M (viscosity 12000-21000 m Pas) *viz.* TH7, TH8 and TH9 were prepared by the same method of extragranulation same which was used for HPMC K4M (batches TH4, TH5 and TH6).

Enteric coating of matrix system

To develop CDDS, formulations were selected in such a manner that it releases more than 70% of the drug at the end of 10 hr. From developed formulations of eudragit L-100, eudragit S-100, HPMC K4M and HPMC K15M, TH5 was selected as it showed desired release pattern (table 2) 70% of the drug was released at the end of 10 hr. The selected formulation TH5 composed of time dependant polymer, HPMC K4M. To avoid variation in drug release due to gastric emptying time, enteric coat which has many other advantages (17) was preferred to avoid contact of tablet with fluid in stomach. For this propose 5% w/v solution of CAP, coating solution was prepared by dispersion of CAP in IPA first by stirring and addition of methyl chloride for 10 minutes and then by stirring for another 20-25 minutes. Solution was filtered through nylon cloth and volume of the solution was measured. Coating was carried out in Kalweka coater (India) by spraying of coating solution with a spray gun on moving tablet bed at speed of 14-15 rpm and was continued till tablets gained weight of around 6%. Composition of enteric coating solution was CAP (5% w/v), methylene chloride (60% v/v) and isopropyl alcohol (40% v/v).

Evaluation of granules, uncoated and enteric-coated tablets

Granules prepared with all above batches were evaluated for percent of the drug content, percent of moisture content, sieve analysis, aerated bulk density (g/mL), packed bulk density (g/mL), percent of compressibility, angle of repose (°) and flow rate (g/sec) (18). Compressed and enteric coated tablets were evaluated for percent of the drug content, average weight (g), dimensions (mm) in terms of length, breadth and thickness, hardness (kg), percent of friability and *in vitro* release profile, as depicted in table 2, and figures 1 and 2.

Content of active ingredient was determined by IP 1996 procedure (15) where tinidazole tablet was dissolved in methanol and absorbance was measured at λ_{max} of 317 nm on UV-Visible spectrophotometer (Jasco V-530- Japan). Disintegration test according to I.P. (Electrolab Ltd.) was performed for enteric coated tablets. Six tablets were first suspended in water for 5 minutes, then for 2 hr in 0.1N HCl. Tablets were observed for coating damages and then suspended in mixed phosphate buffer of pH 6.8 till enteric coats were dissolved. *In vitro* drug release studies were carried out in triplicates using USP dissolution apparatus (Apparatus I, 100 rpm, 37 °C) for 2 hr in 0.1N HCl and continued for 8 hrs in pH 6.8 buffer. At the end of each hour, aliquots were withdrawn and diluted with respective media. Absorbance was measured at 276 nm and 316 nm against 0.1N HCl and pH 6.8 buffer as blank respectively.

Process optimization

Formula which was for reproducibility and scale up studies of TH5 batch is shown in table 4. HPMC K4M and K15M showed satisfactory results with respect to *in vitro* release profile. From nine batches, the TH5 formulation showed desired release pattern where 40% of the drug was released by 3 third hrs and more than 70% of the drug at the end of 10 hrs. This batch was examined for its process reproducibility, release pattern and was scaled up to 250 tablets batch (table 4). In the final formula, weight of PVP was adjusted with MCC and tablet weight was maintained as 0.8 g. Before laboratory scale up, reproducibility of the process was checked. Five

batches of the size of 250 tablets were prepared and coded as TH251, TH252, TH253, TH254 and TH255. Granules and tablets were studied for their physicochemical characteristics (table 5). TH5 batch was enteric coated and evaluated for physicochemical parameters and *in vitro* dissolution profile as shown in tables 6 and 7. These batches were kept for accelerated stability studies according to ICH guidelines.

Bioavailability study of developed enteric coated, uncoated and conventional marketed tinidazole tablets

Bioavailability of tinidazole has been reported (19) and this investigation it was conducted to evaluate performance of the dosage form in normal, healthy adults under standardized conditions (8) by using developed enteric-coated, uncoated and conventional marketed tinidazole tablets of similar strength (Fusigyn: Tinidazole tablet 500 mg, Pfizer India Ltd. Batch no. 220-93051L) on nine healthy volunteers divided in three groups for 24 hrs according to the protocol, by parallel group design involving 3 volunteers per group (first group of subjects received marketed formulation, 2nd group received developed uncoated formulation and 3rd group received developed enteric coated formulation, and were compared with each other). The protocol was approved by Institutional Ethical Committee. Volunteers were subjected to thorough clinical examination, hematological and biochemical investigations such as hemoglobin count, differential count, total white blood cell count, erythrocyte sedimentation rate, random blood sugar, blood urea, serum creatinine, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) prior to inclusion in the study. Tablet was administered after 10 hr fasting. For blood collection, venous cannula was fixed into the antecubital vein under aseptic condition and samples were collected as per scheduled time intervals *viz.* 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, 18, 20 and 24 hrs. At each time point, 8 mL of blood was collected in 15 mL centrifuge tube. Plasma was separated by centrifugation and analyzed by HPLC method (2, 3). Pharmacokinetic parameters like C_{max}, T_{max} and area under curve (AUC) were determined from the plot of plasma concentration (µg/mL) *vs* time (hr) profile curve (5).

High performance liquid chromatography analysis of tinidazole in plasma

HPLC system (Hewlett Packard, HP 1100 series with chemstation software version G2170AA) with UV-Visible detector (278 nm), C_{18} column (Bondapack, 300 × 3.9 mm, particle size 10 µ) was used to analyze tinidazole in plasma using mobile phase- buffer (0.1% triethylamine, pH was adjusted to 2.5 by addition of dilute H_3PO_4) and acetonitrile in ratio of 80:20. Flow rate was adjusted to 1.0 mL/min. Standard curve was constructed for tinidazole in the range of 0.2 to 20 µg/mL in acetonitrile. Tinidazole (0.5 mL) was extracted from plasma with 0.25 mL of 20% perchloric acid. After centrifugation at 3000 rpm, 50 µL of the clear supernatant solution was injected into HPLC column, detected by UV at 254 nm and the data was acquired, stored and analyzed with software. The method was linear ($y = 46763x + 4931$; $r^2 = 1$).

RESULTS AND DISCUSSION

Drug and polymers were standardized and it was found that they have specifications given in official compendia. Different batches of tinidazole were formulated using pH dependant polymer eudragit [eudragit L-100 in concentration range of 10-25% w/w (TL1-TL4) and eudragit S-100 in concentration of 5 and 10% w/w (TS1 and TS2)] and time dependant polymer HPMC [HPMC K4M (TH1-TH6) and HPMC K15M (TH7-TH9) in concentration range of 10-20% w/w] as shown in table 1. Physicochemical parameters of all develop tablet formulation are shown in table 2. Uncoated tablets disintegrated within 15 minutes in water whereas enteric coated tablets disintegrated in buffer pH 6.8 within 5 minutes. Drug release profile of tinidazole tablets is shown in figure 1.

From the data it was found (table 2) that delayed release pattern could be obtained with eudragit L-100, if more than 25% of eudragit L-100 was used. However the formulation would not be cost effective when the ratio of drug to polymer per tablet is taken into consideration and hence, these batches were not carried further. Though eudragit S-100 retarded release of the drug from tablet at 5% and 10% level, but it was difficult to maintain uniformity of the mixture of drug and polymer on large scale and therefore formulations prepared with eudragit S-100 were not considered for further development. pH dependent polymers *viz.* eudragit L-100 and S-100 showed large variation in release profile with minimum pH changes (19). Since pH change in GIT is due to diseases and disorders is unavoidable (6, 7, 20-22), these formulations would not be as effective compared to pH independent polymers and as a result formulations containing pH independent polymers were preferred for further study.

For granules prepared with HPMC K4M (batch TH1, TH2 and TH3) by intragranulation method, it was observed that granules of batch TH2 and

Table 1. Composition of tinidazole controlled release colon specific tablets (Tablet weight=800 mg)

Sr. No.	Batch code	Tinidazole IP (mg)	Eudragit (%)	HPMC K4M (%)	HPMC K15M (%)	MCC IP (%)	Mg stearate IP (mg)	Talc (mg)
1	TL1	500	10	-	-	24.5	12	12
2	TL2	500	15	-	-	19.5	12	12
3	TL3	500	20	-	-	14.5	12	12
4	TL4	500	25	-	-	9.5	12	12
5	TS1	500	05	-	-	29.5	12	12
6	TS2	500	10	-	-	24.5	12	12
7	TH1	500	-	9.75	-	24.5	12	12
8	TH2	500	-	14.63	-	19.5	12	12
9	TH3	500	-	20	-	14.5	12	12
10	TH4	500	-	9.75	-	24.5	12	12
11	TH5	500	-	14.63	-	19.5	12	12
12	TH6	500	-	20	-	14.5	12	12
13	TH7	500	-	-	9.75	24.5	12	12
14	TH8	500	-	-	14.63	19.5	12	12
15	TH9	500	-	-	20	14.5	12	12
16	ETH5	500	-	14.63	-	19.5	12	12

T, Tinidazole; L, Eudragit L-100; S, Eudragit S-100; H, HPMC K4M/ HPMC K15M; -, Not added

Table 2. Physiochemical evaluation of tinidazole controlled release colon specific tablet formulations

Batch No.	% Content of active ingredient	Uniformity of weight (g)	Hardness (kg)	Friability %	% drug release	
					At the end of 2 hrs (in 0.1N HCl)	At the end of 8 hrs (pH 6.8 buffer)
TL1	103.00 (±1.12)	0.797 (±0.010)	8.3 (±0.51)	0.2356	07.63 (±0.20)	94.20 (*5.45)
TL2	99.07 (±2.12)	0.800 (±0.003)	6.1 (±0.97)	0.4851	22.03 (±1.02)	97.68 (*2.10)
TL3	101.64 (±1.32)	0.801 (±0.004)	5.3 (±0.50)	0.5117	14.39 (±5.82)	101.37 (*0.80)
TL4	100.97 (±1.42)	0.794 (±0.001)	5.7 (±0.43)	0.5312	19.54 (±0.94)	98.03 (*1.38)
TS1	95.00 (±5.01)	0.799 (±0.004)	5.3 (±0.51)	0.4670	29.07 (±0.293)	76.65 (*1.49)
TS2	93.27 (±4.40)	0.798 (±0.004)	6.1 (±0.66)	0.3950	19.51 (±0.78)	32.45 (*0.13)
TH1	102.50 (±3.03)	0.853 (±0.010)	4.7 (±0.29)	0.3270	60.04 (±3.31)	95.29 (*0.79)
TH4	101.80 (±2.01)	0.910 (±0.010)	9.6 (±0.29)	0.3980	50.52 (±4.07)	97.96 (*2.493)
TH5	102.56 (±1.23)	0.912 (±0.011)	8.6 (±0.50)	0.3560	36.02 (±2.12)	64.70 (*3.62)
TH6	101.01 (±2.01)	0.820 (±0.005)	4.5 (±0.504)	0.3980	23.38 (±2.246)	44.44 (*1.744)
TH7	96.76 (±5.004)	0.827 (±0.012)	4.1(±0.583)	0.423	32.19 (±3.86)	69.00 (*6.18)
TH8	106.10 (±0.904)	0.828 (±0.004)	4.9 (±0.6633)	0.389	26.33 (±6.50)	52.17 (*0.72)
TH9	100.97 (±1.004)	0.815 (±0.005)	4.6 (±0.374)	0.378	13.09 (±1.76)	29.74 (*1.52)
ETH5	99.89 (±0.125)	0.855 (±0.007)	6.2 (±0.312)	-	0.923 (±0.184)	50.73 (*2.863)

values in bracket represent S.D; T- Tinidazole, EL- Eudragit L-100, TS- Eudragit S-100, H- HPMC K4M/ K15M, E- enteric coated.

Table 3. Composition for scale up studies

Formulation	TH5	
Ingredients	Per tablet (g)	For 250 tablets (g)
Tinidazole IP	0.500	125.00
MCC IP	0.136	34.00
PVP K30 (10% solution)	0.020	5.00
HPMC K4M	0.120	30.00
Mg stearate IP	0.012	3.00
Talc IP	0.012	3.00
Tablet weight	0.800	0.800
Polymer fraction in tablet (%)	15.0	15.00

Table 4. Evaluation of tinidazole granules for reproducibility studies

Property of granules	TH251	TH252	TH253	TH254	TH255
Drug content (%)	104.2	107.0	106.23	105.5	106.07
Moisture content (%)	2.15	1.98	2.19	1.76	2.13
Sieve analysis (%)					
# 36 retained	1.52	0.72	2.01	0.98	0.87
# 60 retained	50.08	19.95	52.43	50.25	52.49
# 85 retained	23.40	25.50	23.49	24.78	26.18
# 100 retained	14.00	13.55	12.92	14.26	13.24
# 100 passed	11.00	10.25	9.13	9.73	7.25
Aerated bulk density (g/mL)	0.4154	0.4589	0.4250	0.4013	0.4100
Packed bulk density (g/mL)	0.5726	0.6310	0.6000	0.5281	0.5023
% Compressibility	27.45	27.27	29.16	23.99	18.36
Angle of repose (°)	28.67	28.67	26.87	26.68	26.56

Table 5. Evaluation of tinidazole tablets for reproducibility studies

Evaluating parameters	**TH251**	**TH252**	**TH253**	**TH254**	**TH255**	**ETH5**
Drug content (%)	103.01	104.03	102.41	104.25	101.01	99.89
Average weight (g)	0.809±0.012	0.808±0.019	0.8067±0.009	0.8088±0.035	0.8058±0.021	0.905±0.010
Thickness (mm)	5.01±0.01	5.09±0.002	5.02±0.004	5.16±0.005	5.12±0.008	0.576±0.044
Hardness (kg)	4.33±0.288	4.00±0.5	4.00±0.5	4.33±0.763	4.50±0.5	10.66±0.287
Friability (%)	0.2672	0.5803	0.2036	0.2563	0.2570	-

Table 6. Dissolution profile of reproducible batches of TH5 and ETH5 formulations

Time (hr)	TH251		TH252		TH253		TH254		TH255		ETH5	
	% drug release	SD	% drug release	SD	% drug release	SD	% drug release	SD	% drug release	SD	% drug release	SD
1	22.26	2.06	26.32	1.26	30.99	4.23	27.20	1.33	27.62	0.75	1.09	0.46
2	29.83	1.46	35.05	1.02	40.88	5.04	37.47	1.84	37.50	0.63	1.41	0.76
3	39.31	1.17	42.88	1.55	50.84	4.94	44.54	1.54	50.61	0.51	30.96	0.52
4	45.88	1.36	46.06	1.69	55.57	5.87	47.99	2.56	54.91	0.50	41.47	1.71
5	55.11	4.71	50.49	2.32	59.37	5.52	51.53	2.35	60.61	0.34	53.83	1.93
6	61.26	11.5	54.70	3.44	60.31	6.52	55.95	1.62	63.64	0.17	59.68	3.67
7	66.96	7.73	58.48	3.24	65.73	7.07	61.41	1.94	69.67	1.98	67.33	3.18
8	70.35	1.77	69.97	2.29	75.11	5.59	66.25	0.93	71.94	4.26	72.26	1.50
9	73.98	2.51	72.77	1.55	79.16	7.38	69.81	0.89	74.06	3.65	75.59	3.38
10	76.95	1.83	78.66	0.42	80.65	6.40	-	-	-	-	79.53	3.48

Table 7. Comparative results of bioavailability study on healthy human volunteers

Time (hr)	Concentration of tinidazole in plasma (µg/mL)								
	Marketed conventional tinidazole tablet			Developed uncoated tinidazole tablet* (TH5)			Developed coated tinidazole tablet* (ETH5)		
	Mean Conc. (µg/ mL)	(±) S.D.	AUC	Mean Conc. (µg/ mL)	(±) S.D.	AUC	Mean Conc. (µg/ mL)	(±) S.D.	AUC
0	0.00	0	0.000	0.00	0	0.000	0.000	0	0.000
1	10.60	3.71	5.300	1.67	0.53	0.837	0.000	0	0.000
2	13.80	1.47	17.503	3.34	0.78	3.344	0.039	0.07	0.019
3	13.58	0.67	31.195	5.04	0.83	7.532	0.997	1.02	0.537
4	13.10	0.76	44.536	6.30	0.89	13.201	1.437	1.06	1.752
5	12.50	0.72	57.339	7.22	1.6	19.964	2.000	1.85	3.473
6	12.07	0.53	69.623	7.41	1.81	27.282	2.483	1.09	5.713
8	10.73	0.41	92.422	6.86	1.59	41.558	2.906	1.72	11.102
10	10.01	0.6	113.160	7.66	1.41	56.076	3.776	0.75	17.783
12	9.61	0.78	132.340	5.88	1.81	69.609	4.524	1.71	26.086
16	7.52	0.8	165.700	4.67	0.96	90.709	5.471	0.17	46.077
20	6.65	0.69	194.050	4.06	1.01	108.171	6.485	0.81	69.990
24	5.59	0.68	218.520	3.26	1.31	122.810	5.766	1.87	94.493
C_{max}	13.80	0.74		7.66	0.45		6.845	0.64	
T_{max}	2.50 hr	0.23		6.00 hr	0.36		20.00 hr	0.84	
$t_{1/2}$	16.84 hr	0.93		12.27 hr	0.69		24.38 hr	0.64	
$(AUC)_0^{\infty}$	348.43	3.21		180.81	2.21		423.28	5.21	

*n=3 volunteers

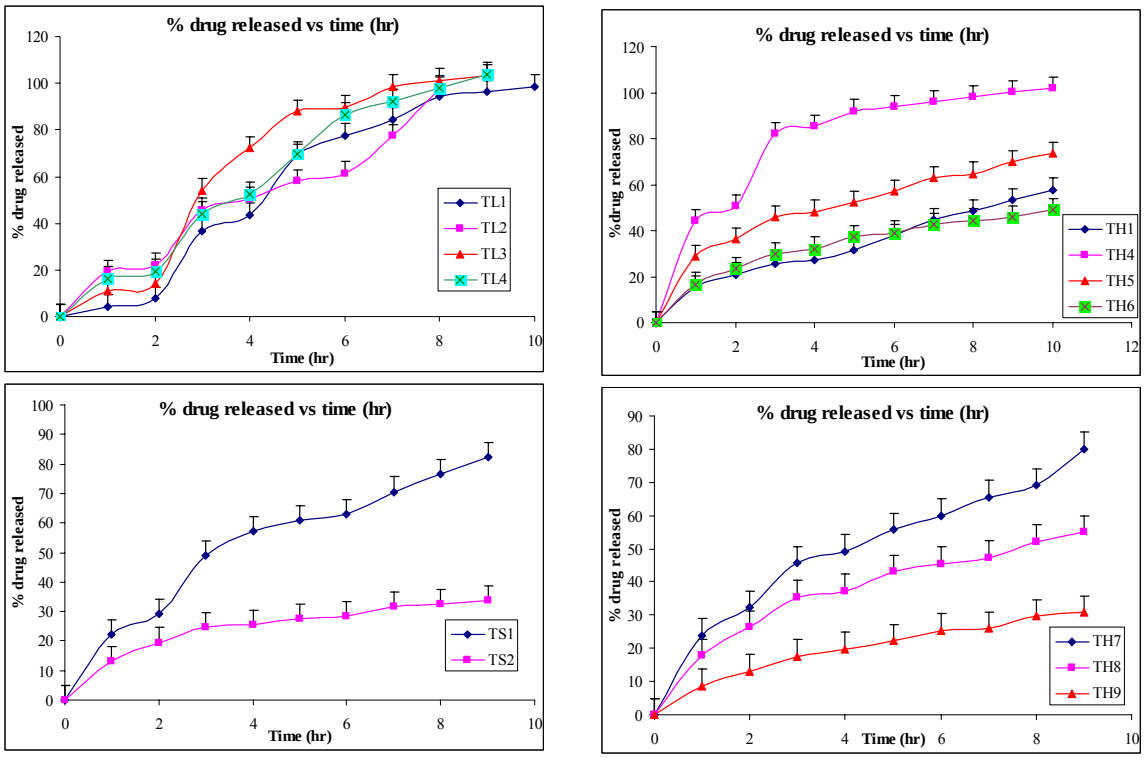

Figure 1. Dissolution profile of tinidazole matrix tablets prepared with (a). Eudrgit L-100; b). Eudragit S-100; (c). HPMC K4M; (d). HPMC K15M

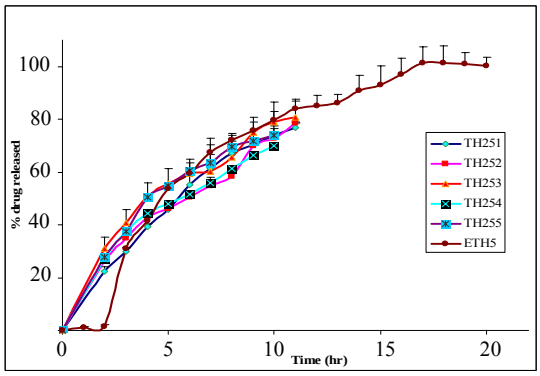

Figure 2. Dissolution profile (in 0.1N HCl for first 2 hr and in pH 6.8 buffer for remaining time) of formulation TH5 subjected to scale up studies and enteric coated batch ETH5

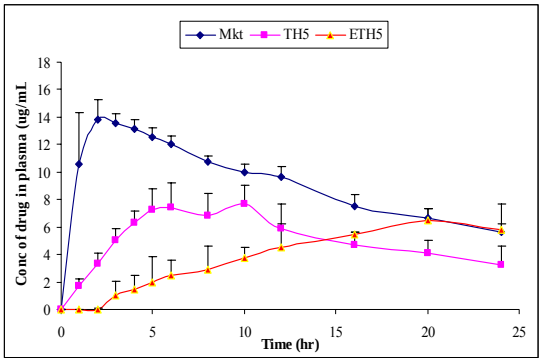

Figure 3. Plot of plasma drug concentration *vs* time for bioavailability study of developed enteric coated, uncoated and marketed conventional tinidazole tablets

TH3 became very hard due to addition of water as binding solvent and it took more than 30 minutes to dissolve in methanol during analysis. Also these batches took more than 4 hrs to dry at 60 °C. Considering all these points, batches TH2 and TH3 were avoided for further studies. From the remaining batches to develop CDDS, formulation was selected in such a manner that it releases more than 70% of the drug from the tablet at the end of 10 hrs. Out of formulations TH1- TH9, TH5 was selected since present of the drug which was released at the end of 2 and 8 hrs were 36.02 and 64.70% respectively (table 2 and figure 1c). Enteric coated formulation TH5 was also studied for physicochemical properties and it released 0.923% and 50.73% of tinidazole at the end of 2nd hr and 8th hr respectively as shown in table 2 and figure 2.

Considering gastrointestinal parameters (pH, volume of fluid in small and large intestine) (23), enteric coated tinidazole tablets containing 10% HPMC K4M were selected for bioavailability studies. Five reproducible batches (TH251-TH255) of granules were evaluated for the percent of the drug content, moisture content, sieve analysis, aerated bulk density, packed bulk density, percent of compressibility and angle of repose and results are given in table 4 and tablets were studied for parameters listed and compiled in tables 5 and 6 and figure 2. These batches were enteric coated (ETH5) and were studied for *in vitro* drug release according to the USP XXIV (24) for 20 hr and tinidazole release from these batches was about 80% at the end of 10 hr whereas 100% release was achieved at the end of 20 hr. Release from the matrix tablet containing swellable polymer was totally dependent on the time of contact of dissolution media and the polymer. It was observed that in stomach there was negligible release of tinidazole and 100% release was observed in colon.

Parallel group design was followed to compare bioavailability of tinidazole from developed enteric-coated tablets (ETH5), developed uncoated tablets (TH5) and marketed conventional tablets. From bioavailability data, it was revealed that tinidazole was released, absorbed and maintained plasma level of 4 µg/mL in colonic region. It was interesting to observe that the marketed formulation showed peak at the end of 2 hr itself as is reported in figure 3 and table 7. Developed enteric coated formulation had slow rate of absorption. There was no drug absorption during first 2 hrs of administration indicating no absorption in the stomach region. Drug was slowly absorbed during next four hours from the intestinal region. Major portion of drug was absorbed after 10 hrs indicating greater bioavailability of the drug at the later portion of the intestine or colon. Tinidazole concentration in plasma was maintained above MIC of 4 µg/mL for 10 to 24 hrs. C_{max} of developed uncoated and enteric coated tablets were lower (7.66 and 6.845 µg/mL respectively) compared to marketed formulation (13.80 µg/mL) which might results in reducing side effects. Results of bioavailability study indicated potential use of controlled release tinidazole formulations for both systemic and local protozoal infection with dose of 500 mg of tinidazole per day.

CONCLUSION

From the data of bioavailability study, the proposed enteric coated tinidazole (500 mg) HPMC K4M tablet per day could be used in place of 3-4 doses of 500 mg tinidazole conventional tablet with better control of drug release for targeted drug delivery which might improves patient compliance and reduces gastric side effects. In summary, time and pH dependent controlled release mechanisms could achieve colonic specific drug delivery of tinidazole following oral administration. Developed CDDS are relatively inexpensive and easy to be manufactured by conventional pharmaceutical

coating technique and is a promising candidates for specific drug delivery to the colonic region, in particular for tinidazole of this study.

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