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FORMULATION DESIGN, *IN-VITRO* AND BIOLOGICAL CORRELATION OF SITE SPECIFIC DRUG DELIVERY SYSTEM FOR DISTAL GASTRO-INTESTINAL TRACT

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ABSTRACT: In the present studies was designed to evaluate oral compression coated tablet formulation to achieve time and site specific drug release system to target lower GIT. A pectin based compression coated tablet formulation of different ratios and concentration of ethyl cellulose and cellulose acetate phthalate were designed and evaluated physico chemical parameters and for tinidazole release, compression coated tablet formulation 7.5% : 7.5% ethyl cellulose: cellulose acetate phthalate showed controlled drug release, were formulation showed no evidence of drug release in pH 1.2, but drug released was in simulated intestinal fluid pH 7.4 after 5 hours, further drug release continued in pH 6.8 i.e. pH of colon for 24 hours and was 87.38% after 24 hour, suited for colon specific drug delivery, on subjecting the selected formulation to *in vitro* microbial disintegration studies, the disintegration started within 4 hours of incubation period indicates that the usefulness of pectin in the formulation, were pectin is microbially hydrolysable and complete disintegration was observed after 16 hours. *In vivo* oral disintegration/ degradation the result was found to be correlated in their disintegration pattern with microbial disintegration. Finally the images of gamma Scintigraphic studies revealed that the disintegration of the tablet formulation on reaching colon.

INTRODUCTION: Most of the conventional drug delivery systems for treating the colon disorders and diseases are failing as the drugs do not reach the site of action in appropriate concentrations. Thus an effective and safe therapy of these colonic disorders and diseases using site specific drug delivery systems is a challenging task to the pharmaceutical technologists.

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To date, oral delivery is still the preferred route of drug administration; Oral administration offers patients less pain, greater convenience, higher likelihood of compliance, and reduced risk of cross infection and needle stick injuries. Thus, formulations of oral drug delivery continue to dominate more than half of the drug delivery market share.

The colonic region of the GIT is one area that would benefit from the development and use of such modified release technologies. Although considered by many to be an innocuous organ that has simple functions in the form of water and electrolyte absorption and the formation, storage and expulsion of faecal material, the colon is vulnerable to a number of disorders including

ulcerative colitis, crohn's disease, irritable bowel syndrome and carcinomas[1]. Targeted drug delivery to the colon, by means of combination of one or more controlled release mechanisms, hardly releases drug in the upper part of the GIT but rapidly releases in the colon following oral administration. Specifically delivering drug to the colon, a lot of benefits would be acquired in terms of improving safety and reducing toxicity when treating local or systemic chronic diseases [2].

As a result new strategies of drug delivery have been developed to overcome obstacles encountered by oral delivery. Among these strategies, colon specific delivery has been extensively studied from the last two decades [3]. In the present work, Stress has been given to check the microbial hydrolysable nature of polysaccharide drug carriers in colon environment. It is well established and known concept that the *in-vitro* drug dissolution studies in colon environment is difficult as fluidity will be minimal and semisolid in nature. So an attempt was made to study the microbial hydrolysable nature of various polysaccharides in presence of resident bacteria of colon to release the drug present in tablet formulation and evaluated the correlation for *in- vitro*, *in-vivo* and microbial degradation studies.

MATERIALS AND METHOD:

MATERIALS: All the materials used in the studies were of analytical grades. Tinidazole drug gift sample from SUN Pharmaceutical Industries Ltd., Ahmednagar. Pectin, Lactose purchased from Sd Fine Chemicals, Mumbai, Himedia Laboratories Ltd., Mumbai, Ethyl cellulose, Cellulose acetate phthalate Qualigens Fine Chemicals Mumbai, Agar media components from Ranbaxy Laboratories, Bombay Research Lab., Pune, ^{99m} Tecnitium-Diethylene Triamine Penta Board of Radiation and Isotope Technology, Mumbai. Equipments used in the studies were UV Spectrophotometer Systronic 119 Model, USP XXIII Dissolution Apparatus Electrolab, Tablet Press (korch CKO,korch Germany), Tablet Hardness Tester Monsanto Campbell Electronics, Mumbai, Tablet Friability Tester Roche, Richi-Rich Pharma, Bangalore.

METHODS:

Preparation of Core Tablet Containing Tinidazole (T_C):

Each core tablet was prepared for further compression coating consisted of ingredients as per **Table 1**. The materials were weighed, mixed and passed through #60 mesh and ensured complete mixing. The thoroughly mixed materials were then directly compressed into tablets using flat and plain punches on tablet press (korch CKO, korch Germany). Tablet quality control tests such as weight variation, hardness, friability, thickness, drug content uniformity and dissolution in different media were performed on the core tablets [4].

TABLE 1: FORMULAE USED TO PREPARE PECTIN BASED TINIDAZOLE TABLETS

Formulation Code	Drug	Pectin	Ethyl Cellulose	Cellulose Acetate Phthalate	Lactose	Total
T_c (mg)	100	50	--	--	--	150
T1(mg)	100	50	7.5%	7.5%	105	300

Preparation of Layered Tablet 7.5%:7.5% Ethyl Cellulose: Cellulose Acetate Phthalate Coated Tinidazole Tablet by Compression Coating (T1):

The core tablets were compression coated with different quantities **Table 1** of coating material containing of half of Pectin inside the core tablet and (Tc) formulation, Tc tablet formulation was layered or compression coated, firstly layered with ethyl cellulose coat and Secondly layered with cellulose acetate phthalate containing another half of the pectin, by using flat and plain punches of specially designed compression coating tablet press (korch CKO, korch Germany) [5, 6]. Tablet was tested for various physicochemical parameters according the pharmacopeial standards.

***In-Vitro* Dissolution Studies:**

Ethyl cellulose coated: cellulose acetate phthalate compression coated tablet (T1) taken in the

separate basket of the dissolution test apparatus Electrolab, TDT-06 P; USP XXXIII standards containing 900ml of dissolution medium. The basket was adjusted to rotate at 40±2rpm. A temperature of 37±1ºC was maintained throughout the experiment. The dissolution process was carried out in different dissolution medium of pH 1.2 for 2 hours and pH 7.4 for subsequent 4 hours and at pH 6.8 for further 24 hours. 10ml of aliquots of samples were withdrawn at predetermined time intervals and were replacing with fresh dissolution medium to maintain sink conditions. The samples withdrawn were suitably diluted if necessary and absorbance was measured at wavelengths 318 nm on Systronics 119 UV visible spectrophotometer[7].

Microbial Degradation Studies of Tinidazole Pectin Based 7.5% (EC) Ethyl Cellulose 7.5% (CAP) Cellulose Acetate Phthalate Compression Coated Tablet (T1) For Microbial Degradation:

Agar media was prepared as given in **Table 2** as per the microbial standards. The media was transferred to petridish and kept for solidification. 0.5 ml of resident colonic bacterial suspension was aseptically transferred into Petridish. The sample was spread over the surface of the agar medium by rotating the petriplate manually or by placing it on the rotating plate disc.

A ditch was made on the centre of petriplate by using 7mm-diameter sterile cork borer and tinidazole pectin based 7.5% EC: 7.5% CAP compression coated tablet (Formulation code-T1) was place, all the procedure was done under strict anaerobic conditions. After 4 hours dissolution study, the intact tablet sample in the basket was transferred to study the microbial degradation/disintegration in petriplates of agar medium containing resident colonic bacteria, and was incubated for different time intervals. At each interval the petriplate were photographed to observe degradation/disintegration rate [8]. Photographs for visual evidence are shown in **Fig.6**.

TABLE 2: PREPARATION OF STERILE MEDIA OF AGAR (M_1)

Composition	M_1
Agar (gm)	2.00
Peptone (gm)	5.0
Beaf extract (gm)	1.5
Sodium Chloride (gm)	5.0
Yeast extract (gm)	1.5
Water (ml)	1000

***In Vivo* Oral Administration Studies:**

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) of Luqman College of pharmacy, Gulbarga with CPCSEA Registration No. 346/CPCSEA.

After 4 hour of dissolution studies the tablet (T1) formulation were intact and fed to the overnight fasted 16 male Albino wistar rats, 230 – 270 g, into the stomach via polyethylene tubing under light ether anesthesia and again kept fasted with free access of water.

Two hundred micro liters of distilled water were administered to rats every 3 h. After each hour two animals were sacrificed and tablet is located in the gut lumen [9]. Its appearance is also checked **Table 6**.

***In Vivo* Gamma Scinthigraphic Studies:**

To the core of tablet (T1) formulation with about 1 MBq of ^{99m}Tc- DTPA *i.e.* ^{99m}Tecnitium-Diethylene Triamine Penta- Acetic acid, (1MBq per 20 mq of resin) [10] was incorporated into (T1) formulation and device is fabricated. This radio labeled tablet was placed into stomach of overnight fasted Wistar rats, 200–270 g, via polyethylene tubing under light ether anesthesia [11]. For the entire duration of study animal was fasted with free access of water. The tablet was visualized using a gamma camera. The gamma scintigraphic imaging started just after dosing and was carried out for 6 hour at 30 minutes intervals [12]. Acquisition was taken for 60 seconds, in supine position. Rats were sedated before immobilization on a mounting chamber on each time of acquisition.

The region of interest (ROI) of stomach and cecum were determined by the scintigraphic images taken just after dosing and at the end of experiments [13], respectively, because those images showed almost the entire GI segment.

RESULTS AND DISCUSSION:

Physico-Chemical Properties for the Tablet Formulations: Evaluation parameters like hardness and friability indicated that the tablets so prepared were mechanically stable and complied with necessary pharmacopoeia specifications.

TABLE 3: PHYSICOCHEMICAL PARAMETERS OF THE TABLETS

Sl.No.	Parameters	Limit	(T_c)	(T_1)
1.	Hardness (kg/cm^2)	--	3.5-6.00	6.00-6.60
2.	Friability	NMT 1%	0.85	0.00
3.	Disintegration time	Uncoated = 15 min	11	---
	(min)	Compression coated = 30 min		
		Compression coated = 60 min		
4.	Weight variation (mg)	< 250 mg – 10%	150±1.0	298±1.02
		250 mg – 7.5%		
		> 250 mg – 5%		
5.	Drug content	95-105%	100.00	98.60

***In Vitro* Drug Release Profile:**

Tablets of Tinidazole meant for colon targeting of drug have sufficiently protects release of drug in the physiological environment of stomach as well as small intestine and majority of drug release occurs in the physiological environment of colon. For (T1) formulation no evidence of drug release in pH 1.2, but a negligible amount of drug released was in simulated intestinal fluid (SIM) pH 7.4 after 5 hours, further drug release studies continued in pH 6.8 i.e. pH of colon for 24 hours and was 87.38% after 24 hour. This indicated that enteric coating protected drug from gastric degradation and further drug release was prolonged up to 24 hours due to increased concentration of Ethyl cellulose and Cellulose acetate phthalate and onset of drug release depends on the polymer coating.

TABLE 4: *IN VITRO* DRUG RELEASE PROFILE FOR 7.5% ETHYL CELLULOSE: 7.5% CELLULOSE ACETATE PHTHALATE COMPRESSION COATED TINIDAZOLE TABLET (T1)

Time (hr)	Absorbance at 318 nm	Conc. in g/ml	Conc. in 900ml (mg)	CLA (mg)	Cum. Drug Released (mg)	Cum% Drug Release
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	0	0	0	0	0
5	0.09	0.718	3.232	0	3.232	2.1049
6	0.03	0.536	4.826	6.464	11.291	7.6477
8	0.161	2.975	26.782	6.491	33.273	22.182
10	0.299	5.545	49.910	6.640	56.551	37.700
12	0.451	8.376	75.385	6.917	82.303	54.868
16	0.546	10.145	91.307	.336	98.643	65.762
20	0.672	12.491	112.42	7.843	120.268	80.178
24	0.731	13.590	122.31	8.468	130.781	87.387

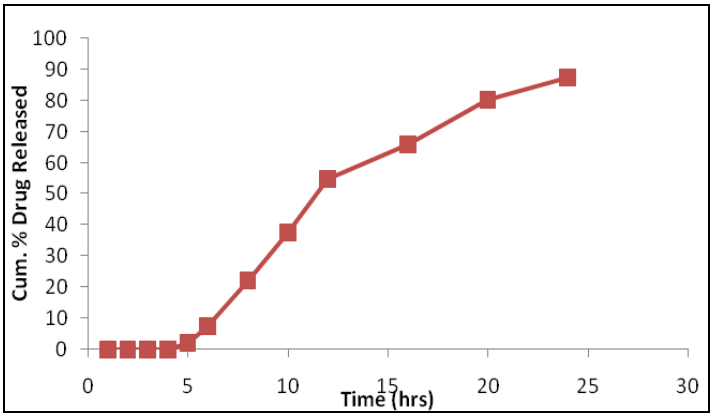

FIG.1: *IN-VITRO* RELEASE PROFILE OF T8 FORMULAIONS

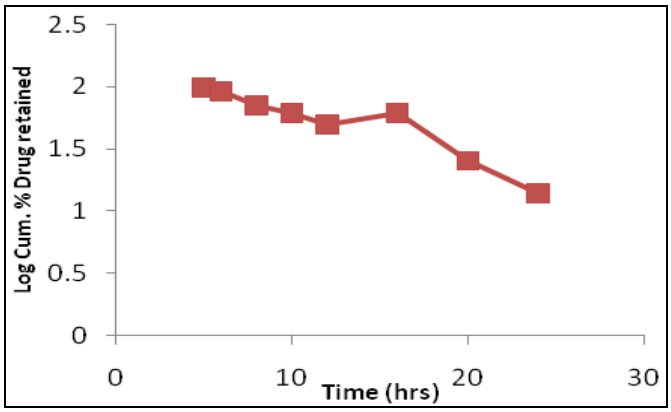

FIG. 2: FIRST ORDER PLOTS OF T1 TABLET FORMULATION

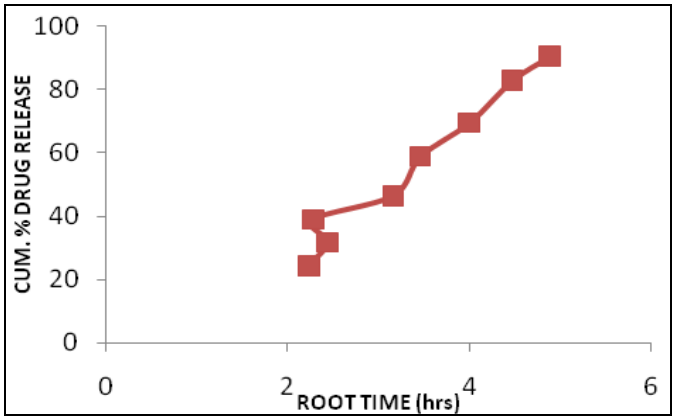

FIG. 3: HIGUCHI MATRIX PLOTS FOR T1 FORMULATION

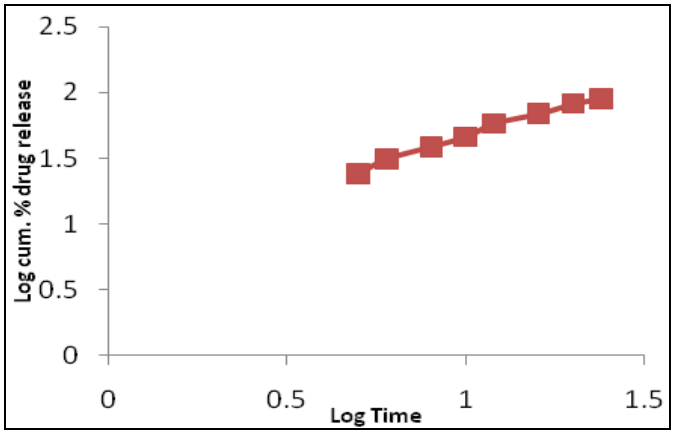

FIG.4: COMPARATIVE PLOTS OF PEPPAS MODEL FOR T1 FORMULATION

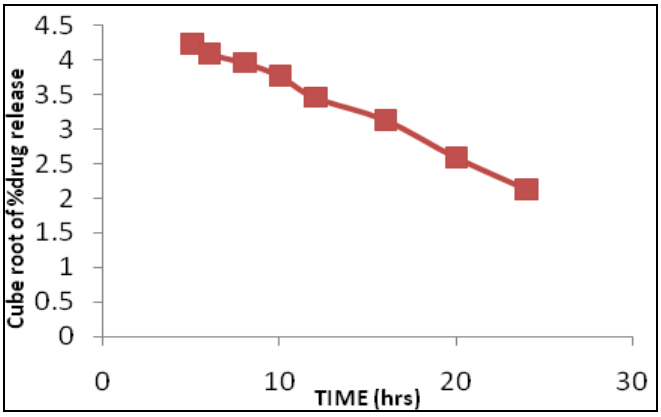

FIG.5: PLOTS FOR HIXSON CROWELL RELEASE STUDIES FOR T1 FORMULATION (ZERO ORDER PLOT)

TABLE 5: KINETIC VALUES OBTAINED FROM *IN-VITRO* RELEASE PROFILE FOR TABLETS

***In- Vitro* Release Profile for Tablets**	**Formulation T1**		
	Slope	**Regression coefficient (r)**	**k value**
Zero order kinetic data	6.754	0.9126	8.5804
First order	-0.0775	0.9821	-0.1743
Higuchi matrix kinetic Data	25.24	0.9912	24.7567
Hixon- rowell model	0.1902	0.9814	-0.0443
Peppas kinetic data	0.9878	24.9719	0.4860

Microbial Degradation Studies of Tinidazole Pectin Based Tablet (T1) Formulation: After 4 hours dissolution studies tablets (T1) formulation were subjected to microbial degradation studies in presence of resident colonic bacteria under anaerobic conditions. Results revealed that tablet formulations degradation started after 2 hours of incubation and complete degradation was observed within 16 hours. Microbial degradation studies gave satisfactory results as can be observed from **Fig.6**.

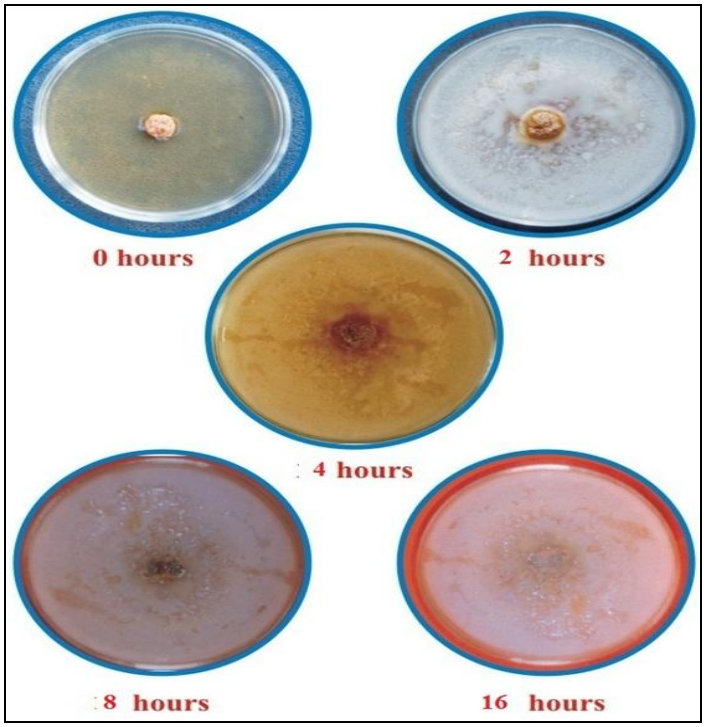

FIG. 6: MICROBIAL DEGRADATION STUDIES FOR TINIDAZOLE-PACTIN BASE TABLET (T1) FORMULATION AT VARIED TIME INTERVAL IN AGAR MEDIA.

In Vivo Oral Administration Study:

When the Tablet formulation (T1) was given orally to overnight- fasted rats, it was found that it could retain its solid integrity in the stomach as well as intestine. Tablet even found intact in the caecum after fifth hour of administration, indicating that it just arrived. On the sixth hour after administration, tablets were found in disintegrating condition. At seventh hour tablet was totally disappeared depicting as disintegrated completely. **Table 6** details the observations.

TABLE 6: *IN VIVO* ORAL ADMINISTRATION STUDIES

Time in hours	Location of tablet		Integrity of tablet	
	Group 1	Group 2	Group 1	Group 2
1	Stomach	Stomach	Intact	Intact
2	Duodenum	Duodenum	Intact	Intact
3	Jejunum	Jejunum	Intact	Intact
4	Ileum	Ileum	Intact	Intact
5.	Caecum	Caecum	Slight erosion	Slight erosion
			Partially degraded	Partially degraded
6.	Caecum	Caecum		
7	Colon	Colon	Disappeared	Disappeared
8	Colon	Colon	Disappeared	Disappeared

In Vivo Gamma Scintigraphic Study:

Gamma scintigraphy using radio labeled tablets **(T1)** formulation was found to be correlating with those of the *in vitro* data. Images relating to Gamma Scintigraphic evaluation are shown in **Fig.7**. Initial images can be hypothesized to be in stomach and later in intestine. Intactness was very clear in those images. As images were taken one in every 30 minutes, the GI transit time can't be calculated accurately. The reason for taking images less frequently, is to minimize the effect of anesthetics on gastric motility, moreover keeping the animal immobilized by stretching on the mount table in supine position may also alter the normal physiology.

Caecum the region of interest comes close to the right hip joint when animal is in supine position[14]. When the radio labeled tablet arrives in the caecum it stays for some time and in fasted condition retention time further increases.

Devised radio labeled (T1) tablet formulation was found to arrive in the colon within 6th hours of post administration. After commencement of disintegration shape of the caecum was arrived to be visible, as radioisotope technetium complexed with DTPA resin, it remained in the confines of lumen. The data so obtained proves the functionality of this novel colon targeted device. The gastric emptying time was less than 1.2 hours. Caecum arrival time was less than 6 hours post-dose. Initial disintegration was found around 6.5 hours and complete disintegration in 7 hours.

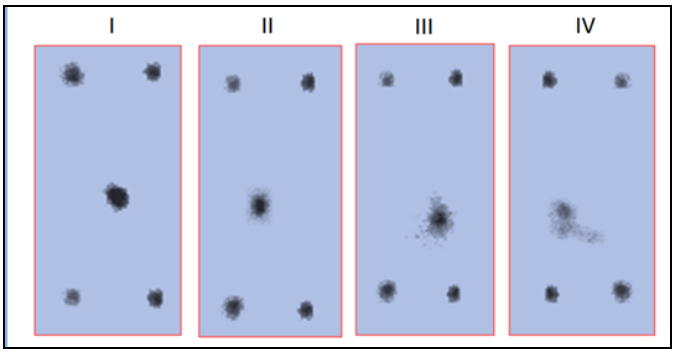

FIG. 7: GAMMA SCINTIGRAPHIC IMAGES OF *IN VIVO* DISINTEGRATION

Spots at all four corners are the anatomical markers indicating two upper 'shoulder joints' and two lower 'hip joints'. (I) tablets in the stomach, (II) in the intestine, (III) in the caecum and (IV) tablet after disintegration in colon.

CONCLUSIONS: The given set of ingredients used in the tablet formulation (T1) proved that the perfect correlations between *in-vitro* studies: Microbial degradation studies**:** *in-vivo* studies: Gamma scintigraphy studies. As the in-vitro drug release revealed that the polymer used in the studies holds the drug release for 5 hours and showed perfect enteric coated properties.

After 4 hours dissolution (T1) formulation tablet subjected to microbial disintegration/degradation studies, Tablet (T1) formulation disintegration started within 4 hours of incubation indicates that

the usefulness of pectin in the formulation, were pectin is microbial hydrolysable, and complete disintegration was observed after 16 hours.

In vivo oral administration study showed that on the sixth hour after administration, tablets were found in partial disintegrating and at seventh hour tablet was totally disappeared depicting as disintegrated completely, indicates that *in vivo* and *in vitro* correlations of disintegration.

Scintigraphic evaluation of most promising formulation T1 showed most satisfactory results that indicates strong agreement between the in vitro /in *vivo* data of disintegration of tablet formulation.

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