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In-vitro Drug Release Studies of 5-Fluorouracil from Novel Enteric Coated Capsules Utilizing Combined Approaches of pH-dependent and microbial triggered biodegradable polysaccharides for Colon Specific Delivery

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ABSTRACT

The objective of present study is to design a novel enteric-coated colon targeting drug delivery system of 5-fluorouracil (5-FU) using biodegradable guar gum as a carrier for colorectal cancer treatment. Formulation matrix containing 30% guar gum was prepared and coating was done using polymers of hydroxyl propyl methyl cellulose (HPMC) for inner hydrophilic coating and Eudragit®L/S-100 for outer enteric coating in different ratio (2:4, 3:2, 3:4 and 4:3). The prepared formulations were subjected to in-vitro drug release studies in various simulated gastric and intestinal fluids, were found gastro resistant for 2 h at pH 1.2 and further 3 hr at pH 7.4, since they released only less than 10% of drug. Furthermore, the release studies was carried out in absence (control) and presence of simulated colonic fluid media containing 2 and 4% w/v rat caecal content. The results obtained after enzyme induction for the period of 2, 4 and 6 days revealed significant release profile compared to control at the end of 24 h studies. Further, report suggested that guar gum was biodegradable and susceptible to the colonic microfloras under anaerobic environments. Scanning electron microscope (SEM) report of surface coated formulations illustrated that HPMC provided rough surface for good adhesion to enteric Eudragit®L/S-100 films over the plain gelatin. DSC thermogram showed no possibilities of interferences between drug and polymers used during formulation development. Therefore, it

can be concluded that guar gum is a promising potential carrier for targeting 5-FU in the vicinity of colon in order to treat colon cancer effectively.

Key Words: Rat caecal content medium; pH-sensitive polymers; biodegradable Guar gum matrix; *In-vitro* and *in-vivo* drug dissolution studies; lag time.

INTRODUCTION

Targeted drug delivery system is gaining extensive importance to treat colonic abnormalities in the field of pharmacotherapy. It has been reported that the colon is beneficial for local treatment of number of pathologies such as colorectal cancer, chroh's disease, inflammatory bowel disease and amoebiasis [1]. Further, colon does not inherently possess the ideal anatomical and physiological features than upper gastrointestinal tract, but is the site having negligible brush-border membrane peptidase activity, longer retention time (20-30 h), highly responsive to poorly absorbed drug enhancers and somewhat less hostile recognized environments [2, 3]. Consequently, colon would be a promising site for both local and systemic drug delivery [4]. Conventional dosage forms were not efficient in delivering drug to colon in appropriate concentration due to being absorbed or degraded by the hostile upper gastrointestinal tract. The colon was friendlier because of less acidic or enzymatic activity and offers almost non-varying neutral pH [5, 6]. Over the last few years, various approaches have been utilized for oral delivery of drug(s) in order to achieve colon specific drug delivery system [7-9] which include time dependent delivery [10], pH sensitive polymer coatings [11, 12], microbially triggered enzymatic degradation by colonic bacteria [13, 14], prodrug approach based delivery [15] and pressure controlled release systems [16, 17].

It has been suggested that the gastrointestinal pH progressively increases from stomach to colon (pH 2- 8), however, pH decreases significantly from the ileum to colon [18]. Further, it has been evidenced that pH-dependent targeting system and time-dependent delivery system shows poor site specificity due to large variations in pH and gastrointestinal transit time [19, 20]. Furthermore, it has been reported that the coated formulations are protected in the stomach and proximal part of small intestine, indicating good site specificity [21].

Guar gum is a polysaccharide consists of linear chains of (1→4)-β-D- manopyranosyl units with ά-D- galactopyranosyl units attached together by (1→6) linkages, which are derived from the Cyamopsis tetragonolobus seeds [22]. The polysaccharide is hydrophilic in nature that swells to form viscous gel like mass on absorption of dissolution fluids or gastrointestinal fluids. It reduces drug release from the system as well /and additionally highly susceptible to degradation by the colonic microfloral environment [23]. Its hydration and viscosity is unaffected over a wide pH range in the environment of dissolution medium. The results of the *in-vitro* studies carried out in our earlier work suggested that the formulations containing 30% guar gum concentration are best suitable for colon targeting which gets completely disintegrated in the simulated colonic fluid without being released significantly in the upper gastrointestinal tract in comparison to matrix containing 20 or 40% of guar gum were found ineffective [24].

The colonic micro floras were recognized as preferred triggering components on designing of the colon specific delivery systems to achieve greater site specificity. The colon consist of more than 400 bacterial species having population of 10^{11}-10^{12} CFU/ ml namely Bacteriodes, Eubacterium, Lactobacillus, Bifidobacterium etc. responsible for fermentation and degradation of plant polysaccharides for dietary sources [25-27]. The responsible enzymes triggering the polymer degradation include B-xylosidase, β-D-glucosidase, β-D-galactosidase β-D-fucosidase [28, 29]. Further, it has been documented that to overcome certain limitations of conventional USP dissolution testing procedure for evaluation of the colon specific delivery systems, rodent's caecal contents are being utilized more commonly as an alternative dissolution medium which mimics with the human colonic microfloras.

Therefore, in the present experiment it was hypothesized that to develop a combined approach of pH-dependent enteric coating over the delivery system and matrix containing polysaccharide carrier that are exclusively biodegradable by microbial floras of colon.

MATERIALS AND METHODS

Materials

5-Fluorouracil was received from M/s. Shalaks Pharmaceuticals (P) Ltd, New Delhi (India). Eudragit®L and S-100 was donated by Rohm Pharma, Darmstadt (Germany) and HPMC was supplied by Colorcon Asia Pvt. Ltd., Goa (India). Guar gum (viscosity of 1% aqueous dispersion is 125 cps; particle size < 75μm) were procured from Dabur Research Foundation, Delhi (India) of USNF quality and Hard gelatin capsule sizes#2 were obtained from Sunil Health Care Ltd., Rajasthan (India). Diethylene triamine penta acetic acid (DTPA) was obtained from Board of Radiation and Isotope Technology (BRC) Mumbai and ^{99m}Technitium was collected at Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal (India). All other reagents and organic solvents used were of analytical/ pharmacopoieal grade from commercial suppliers.

Preparation of impermeable cross-linked hard gelatin capsule (HGC)

The body and cap of emptied hard gelatin capsule (size#2) were separated and the capsule's body portion was taken in a dessicator containing 25 ml of 15% (v/v) concentration of formaldehyde. The capsules (a batch of 100 in number) kept over wire mesh were exposed to formaldehyde vapors in the tightly closed dessicator for time period of 10 h and simultaneously dried at 50°C for 30 min in an hot air oven for ensuring complete cross-linking reactions between formalin vapors and gelatin. Then exposed bodies were air dried to facilitate removal of residual formalin vapors at room temperature. These cross-linked bodies was capped with the remaining untreated caps portion and stored in a sealed amber colored glass container [30-32].

Preparation and coating of colon targeting delivery capsules (CTDC)

The formulation matrix in all cases consisted of 10 mg 5-FU drug with 30% guar gum was plugged into hard gelatin capsule (size#2) and rest of the volume was adjusted with inert lactose. In all the cases the total weight of the powder mass was maintained up to 150 mg. The joint of capsule's body and cap was sealed with a small amount of 5% w/v ethyl cellulose ethanolic solution. Thereafter each batch of the capsules was coated for inner coating by HPMC (hydrophilic layer) and outer coating with Eudragit®L/S-100 (enteric layer) using dip coating method into the polymeric solution of HPMC and Eudragit®L/S-100 to ensure the formation of

a uniform and thin covering over the capsule. In order to enhance the elasticity of Eudragit®L/S-100 film, 1.25% of dibutyl phthalate as plasticizer was added to the coating solution. For each polymeric solution coating of capsules was made with different thickness ratios of HPMC: Eudragit®L-100 (EdL-100) into 4 batches of CTDC-1 to 4 (2:4; 3:2; 3:4 and 4:3) and with HPMC: Eudragit®S-100 (EdS-100) into another 4 batches of CTDC-5 to 8 (2:4; 3:2; 3:4 and 4:3) respectively by dipping twice, thrice and four times in each coating solutions at room temperature. The film was allowed to dry with the help of dryer with an inlet temperature of 35-40ºC and stored in well-closed container for further studies. The characteristics of coating polymers and their standard operating conditions were presented in Table 1.

Drug content determination

The test was performed with formulations CTDC-1 to 8 by assaying them individually according to USP limits. The capsule was crushed and dissolved in phosphate buffer saline solution (pH 7.4) and volume made up to 100 ml in the volumetric flask. A 0.1 ml aliquot was taken out and volume made up to 10 ml with PBS (pH 7.4) solution and filtered through Whatman No.1 filter paper. The absorbance and percent drug content of the filtrate was recorded at λmax of 265.4 nm for 5-FU with the help of Double Beam UV-Visible spectro-photometer (Shimadzu).

Table 1: Formulae of coating solution and standard operating conditions

Coating layer	Inner layer (Hydrophilic polymer layer)	Outer layer (Enteric polymer layer)
Composition of coating solution (w/v %)	HPMC (4.5%) Ethanol (23%) H_2O (71.5%)	Eudragit®L/S-100 (15%) Ethanol (100%) + Dibutyl phthalate (1.25%)
Operating condition	Simple stirring or by shaking on the shaking table at room temperature (25^{o}C)	

Table 2: *In-vitro* release profile of 5-FU from colon targeting delivery capsules (CTDC) having different coating ratios in simulated GI fluids at pH 1.2 and 7.4.

Formulation Code	Cumulative percent drug released 1 hr	2 hr	3 hr	4 hr	5 hr
CTDC-1	-	-	-	2.1±0.06	5.4±0.37
CTDC-2	-	-	1.3±0.04	3.2±0.15	6.4±0.32
CTDC-3	-	-	-	2.3±0.07	6.0±0.31
CTDC-4	-	-	1.7±0.07	3.3±0.16	6.8±0.35
CTDC-5	-	-	-	1.0±0.02	2.6±0.06^{a,b,c,d}
CTDC-6	-	-	-	2.0±0.05	3.9±0.32^{a,b,c,d}
CTDC-7	-	-	-	2.3±0.19	3.2±0.21^{a,b,c,d}
CTDC-8	-	-	1.6±0.09	2.7±0.13	5.0±0.37^{a,b,c,d}

All the values are expressed in Mean ± SD. ^{a}P<0.05 compared to CTDC-1, ^{b}P<0.05 compared to CTDC-2, ^{c}P<0.05 compared to CTDC-3 and ^{d}P<0.05 compared to CTDC-4 (One-way ANOVA followed by Student Newmann keuls test).

***In-vitro* drug release studies**

The ability of guar gum based enteric-coated formulations to remain intact in the bio-environment of stomach to small intestine was assessed by conducting drug release studies mimicking stomach to colonic pH and transit respectively. The release studies were performed using USP XXIII Dissolution Rate Test Apparatus (Apparatus 1, 100 rpm, 37±0.5ºC) in simulated gastric fluid (SGF) and intestinal fluid (SIF) to assess the effect of different coating and their degradation levels on drug release profile of the formulations CTDC-1 to 8 respectively. Initially the capsules were tested for drug release in 900 ml of SGF containing 0.1 M Hydrochloric acid (pH 1.2) as the average gastric emptying time about 2 h after which the pH of the dissolution medium was adjusted to pH 7.4 containing Sorensen's phosphate buffer solution and tested for 3 h time interval continuously as the average small intestinal transit time is about 3 h [33]. At predetermined time intervals 2 ml samples were withdrawn and replaced by an equal volume of fresh medium to adjust the sink conditions. Samples were filtered, diluted and assayed at each interval for 5-FU content released at λmax of 265.4 nm in SGF and SIF media, respectively (Table 2).

***In-vitro* drug release in presence of rat caecal content medium (RCCM)**

In order to assess the susceptibility of guar gum affecting the performance of colon specific delivery systems triggered by colonic bacteria, *in-vitro* drug release studies were investigated in the presence of rat caecal contents [34]. It has been utilized as an alternative dissolution studies to overcome the limitations of conventional dissolution testing because of similarity of human and rodent colonic microfloras [35, 36]. Healthy adult Wistar albino rats aged 2-3 months and weighing between 150-200 g were used for the experimental study. The Institutional Animal Ethical Committee of University approved the study protocol.

To induce the enzyme that are responsible for biodegradation of guar gum polysaccharides in caecum of the large intestine, albino rats were incubated with Teflon tubing and 2 ml of 1 % w/v solution of guar gum in water was administered directly into the stomach region via oral cavity. The treatment was repeated for Day 2, 4 and 6 in different sets of animals for varying enzymatic inductions. Rats were sacrificed before 30 min of commencing drug release studies and the caecum was exteriorized for content collection. The caecal content (anaerobic nature) were immediately transferred into buffer saline solution (pH 6.8) to obtain an appropriate 2 % and 4% w/v concentration solution which was previously bubbled with nitrogen gas to maintain an anaerobic environment [37, 38].

The drug release studies were performed using USP dissolution test apparatus of basket type (100 rpm, 37±0.5ºC) in sealed anaerobic conditions with modifications in the procedure was done. The experiment was carried out in 250 ml beaker containing 200 ml caecal dissolution media with continuous nitrogen gas supply was kept immersed in water bath for the dissolution test apparatus. The formulations which was subjected previously to *in-vitro* release studies in SGF (pH 1.2) for 2 h were then taken along with 150 ml was added 50 ml of 0.2 m trisodium phosphate to made simulated colonic fluids (pH 6.8) and then finally immersed with 2% rat caecal content in dissolution medium to give final dilutions of 200 ml capacity. At different time intervals, 2 ml sample media was pippeted out regularly and compensated with freshly prepared SCF (pH 6.8) with same amount and the studies was continued till completion of 24 h. The withdrawn samples after volume made up to 5 ml was filtered and was quantified using UV-

Spectrophotometer. The same experiment was repeated with 4% w/v rat caecal content medium (RCCM) for comparative studies of dissolution media. All the studies were carried in anaerobic environment by continuously supplying nitrogen gas into the dissolution media apparatus [39, 40]. Mean cumulative percent drug release was noted and presented in Fig. 1 to 4 respectively.

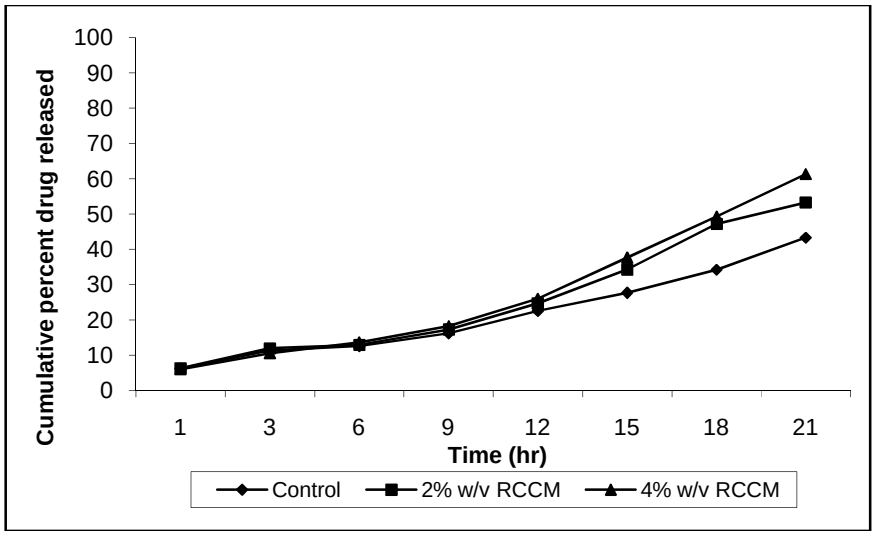


Fig. 1: *In-vitro* release profile of 5-FU from CTDC-5 in SCF (pH 6.8) having absence and presence of 2 and 4% w/v rat caecal content medium obtained without enzyme induction. All the values are expressed in Mean ± SD (One-way ANOVA followed by Student Newmann keuls test).

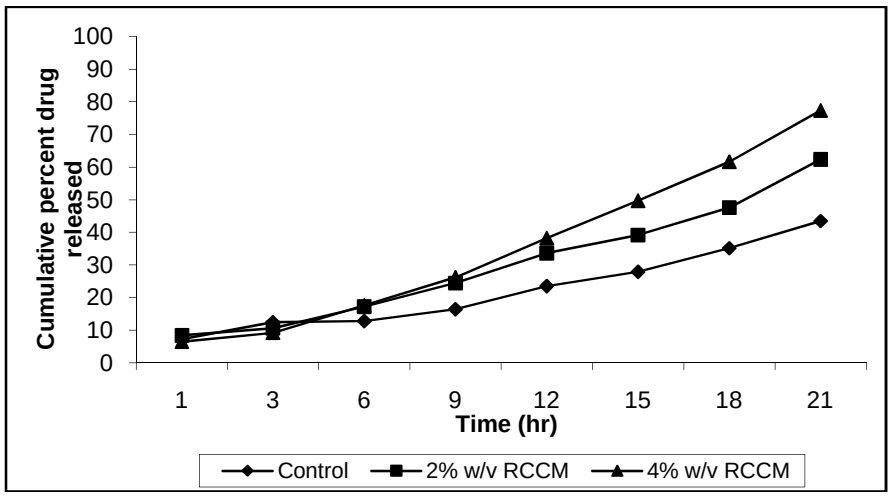


Fig. 2: *In-vitro* release profile of 5-FU from CTDC-5 in SCF (pH 6.8) having absence and presence of 2 and 4% w/v rat caecal content medium obtained after 2 days of enzyme induction. All the values are expressed in Mean ± SD (One-way ANOVA followed by Student Newmann keuls test).

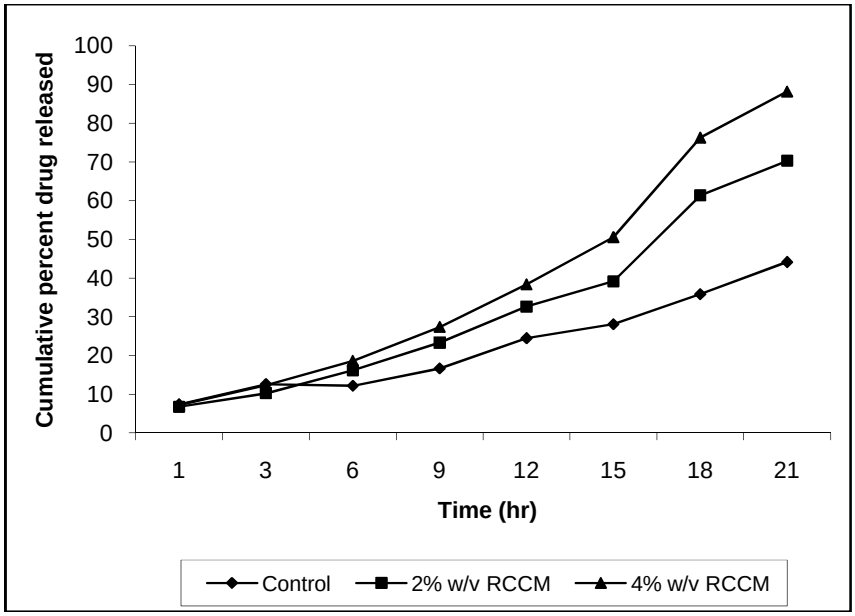


Fig. 3: *In-vitro* release profile of 5-FU from CTDC-5 in SCF (pH 6.8) having absence and presence of 2 and 4% w/v rat caecal content medium obtained after 4 days of enzyme induction. All the values are expressed in Mean ± SD (One-way ANOVA followed by Student Newmann keuls test).

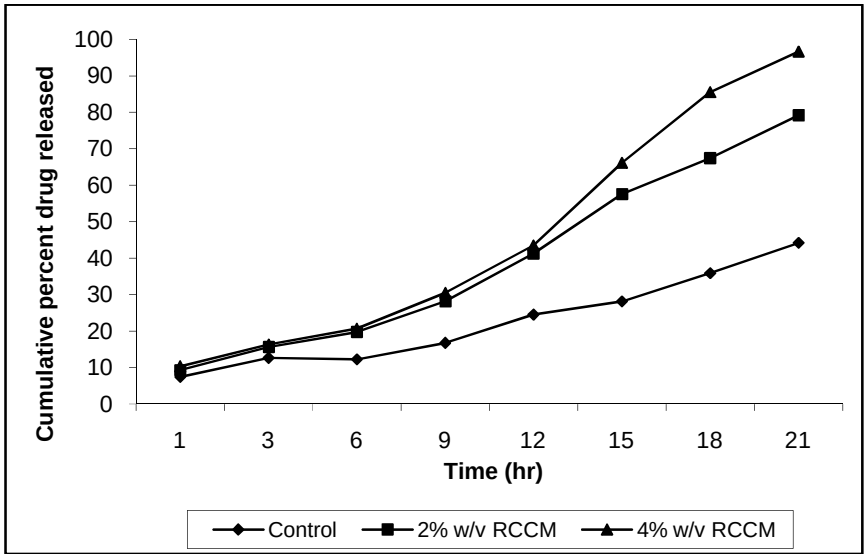
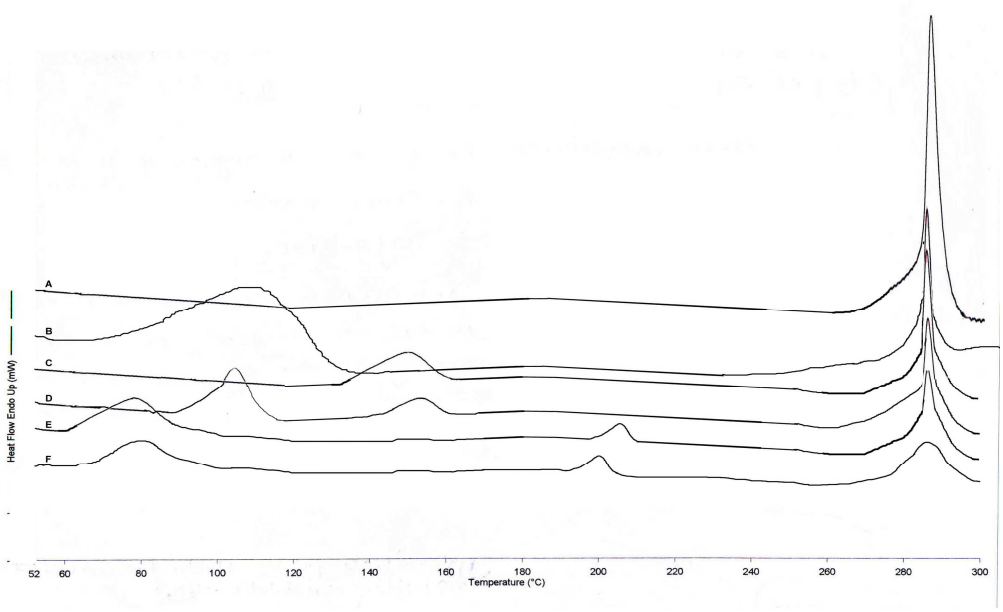


Fig. 4: *In-vitro* release profile of 5-FU from CTDC-5 in SCF (pH 6.8) having absence and presence of 2 and 4% w/v rat caecal content medium obtained after 6 days of enzyme induction. All the values are expressed in Mean ± SD (One-way ANOVA followed by Student Newmann keuls test).



DSC analysis of powdered formulation mixtures (Perkin Elmer, USA)

Differential Scanning Calorimetry (DSC) study was undertaken to detect any possibilities of interaction takes place between drug and excipients during the formulation development, which affects the compatibility of formulations. Samples (2-6 mg) were placed in flat-bottomed aluminum pans and hermetically sealed. The probes were heated from 50ºC to 300ºC at rate of 10ºC/ min under nitrogen atmosphere (50ºC/ min). Thermogram of sample mixtures was obtained as shown in Fig. 5.

Stability studies

Stability studies were conducted for the potential formulation in order to access their long-term stability [41]. The sample was stored at 40ºC/ 75% relative humidity (RH) for 6 month periods to analyze for any change in physical appearance, color, and residual drug content and percent drug release characteristics. Further, the percent drug release studies were also carried out in 4% w/v rat caecal content medium after storage at 40ºC / 75% RH for 6 month periods (Table 3).

Statistical analysis

The results are expressed as Mean ± S.D. The statistical significance was determined by One-Way Analysis of Variance (ANOVA) followed by *Post-hoc* Student Newman Keuls test except for stability studies. Further, the Student-t test was performed for stability studies of the formulation. $P < 0.05$ was considered to be statistically significant.

Table 3: Percent of 5-FU released from optimized formulations in various simulated GI fluids of 0.1 M HCl and PBS 6.8 pH containing 4% rat caecal content before and after storage at 45ºC/ 75% RH for 6 months.

Time (h)	Dissolution medium	Percent of 5-FU released from optimized formulation (CTDC-5)	
		Before storage	After storage
0	1.2 pH (900 ml)	0	0
1	PBS (6.8 pH) containing 4% w/v rat caecal content (200 ml)	1.35±0.21	1.72±0.19
2		2.84±0.32	2.61±0.38
3		5.46±0.36	5.21±0.41
6		19.82±0.81	20.26±0.84
9		32.71±1.46	31.47±1.42
12		40.38±1.59	40.16±1.63
15		52.72±1.95	52.91±1.92
18		61.35±2.13	62.39±2.25
21		68.56±2.37	68.47±2.30
24		94.26± 2.94	93.5±2.89

All the values are expressed in Mean ± SD (One-way ANOVA followed by Student Newmann keuls test).

RESULTS

The results of the *in-vitro* drug release studies carried out for 5-FU containing different concentration of guar gum coated with EdL/ EdS-100 and HPMC in simulated gastric (pH 1.2) and small intestine (pH 7.4) are shown in Table 2. In all the cases the formulations were found intact during 2 hr studies at pH 1.2. The results reported that the formulations CTDC-2, 4 and 8 showed significant releases while others were intact during first 3 hr of dissolutions. This included 2 hr dissolution at a pH of 1.2 followed by 1 hr dissolution at pH of 7.4. This showed that drug released was highly retarded on coating with EdS-100 in comparison to EdL-100 coating. Also by reducing the enteric coating i.e. EdL/ EdS-100 in comparison to inner HPMC coating ratios the initial drug release was not significantly affected for first 3 hr but the total percent drug release was increased. However, there was a considerable swelling of formulations was observed more in formulation with increased HPMC coating layers which can be explained on the basis of its hydrophilic nature.

Moreover, the dissolution studies were further extended up to 5 hr i.e. above solubility pH of the enteric polymers at pH of 7.4 in order to simulate the small intestinal conditions. Visual observation revealed small flakes of coatings occurred about 3.5 h from the beginning of the release experiment. When the same formulations were carried out continuously to *in-vitro* drug release study the matrix capsules coated with HPMC: EdL-100 after 5 h of dissolution showed significant release profile compared to the formulations coated with HPMC: EdS-100. The release of drug from the enteric-coated capsules can be explained by the pore formations and bursting/ flake formation of the coat due to presence of high alkaline pH of dissolution media. As

the pH of solubilization of EdL-100 is 6 and that of EdS-100 is 7.0, EdL-100 gets dissolved first and form pores, at pH 7.4. Furthermore, the release of 5-FU was a function of the thickness of coating i.e. the release being higher the thinner the enteric coating. Additionally the HPMC layer resulted in capsules with higher drug release due to enhanced hydrophilicity of the coatings. It was noticed from the results that, a formulation with EdL-100 coatings gave a too early release, while EdS-100 coated impeded drug release during 3 hr studies at the pH of 1.2 and 7.4. Such release could be due to the entrapped drug nearer to surface that was dissolved and diffused out into the medium after swelling of the formulations without the influence of azoreductase enzymes. The release of less than 10% drug in simulated gastric (pH 1.2) and small intestinal (pH 7.4) fluids indicated the ability of enteric-coated capsules for specific delivery of drugs to the colon.

The percent cumulative *in-vitro* drug released profile of 5-FU from CTDC-5 formulation containing 30% guar gum with rat caecal content (2 & 4% w/v) and without rat caecal content (Control) is depicted in Fig-1. Statistical analysis by One way ANOVA revealed that there was insignificant difference among groups [$F(2, 15) = 0.71$, $P>0.05$] at the end of 1 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 did not show any significant release profile at the end of 1 h study in both 2 % and 4 % RCCM compared to control medium. The similar trend was observed in 3 h [$F(2, 15) = 0.52$, $P>0.05$], 6 h [$F(2, 15) = 0.86$, $P>0.05$], 9 h [$F(2, 15) = 0.78$, $P>0.05$] and 12 h [$F(2, 15) = 0.67$, $P>0.05$]. Further, statistical analysis by One way ANOVA revealed that there was significant difference among groups [$F(2, 15) = 88.3$, $P<0.05$] at the end of 15 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was no significant change in release profile for CTDC-5 formulation in between 2% and 4% w/v RCCM. The similar trend was observed in 18 h [$F(2, 15) = 75.48$, $P<0.05$] study. Furthermore, statistical analysis by One way ANOVA revealed that there was significant difference among groups [$F(2, 15) = 73.7$, $P<0.05$] at the end of 21 h study for CTDC-5 formulation. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 formulation showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was significant increased release profile for CTDC-5 formulation in 4 % w/v RCCM compared to 2 % RCCM.

Fig-2 illustrates the percent cumulative *in-vitro* drug released profile of 5-FU from CTDC-5 formulation containing 30% guar gum with rat caecal content (2 & 4% w/v) and without rat caecal content (Control). Statistical analysis by One way ANOVA revealed that there was insignificant difference among groups [$F(2, 15) = 0.79$, $P>0.05$] at the end of 1 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 did not show any significant release profile at the end of 1 h study in both 2 % and 4 % RCCM compared to control medium. The similar trend was observed in 3 h [$F(2, 15) = 0.75$, $P>0.05$], 6 h [$F(2, 15) = 0.68$, $P>0.05$]. Further, statistical analysis by One way ANOVA revealed that there was significant difference among groups [$F(2, 15) = 88.1$, $P<0.05$] at the end of 9 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was no significant change in release profile for CTDC-5 formulation in between 2% and 4% w/v RCCM. The similar trend was observed in 12 h [$F(2, 15) = 74.3$, $P<0.05$] study. Furthermore, statistical analysis by One way ANOVA revealed that there was significant difference among

groups [F (2, 15) = 73.2, P<0.05] at the end of 15 h study for CTDC-5 formulation. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 formulation showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was significant increased release profile for CTDC-5 formulation in 4 % w/v RCCM compared to 2 % RCCM. The similar trend was observed in 18 h [F (2, 15) = 112.3, P<0.05] and 21 h [F (2, 15) = 102.3, P<0.05] study.

The percent cumulative *in-vitro* drug released profile of 5-FU from CTDC-5 formulation containing 30% guar gum with rat caecal content (2 & 4% w/v) and without rat caecal content (Control) is depicted in Fig-3. Statistical analysis by One way ANOVA revealed that there was insignificant difference among groups [F (2, 15) = 0.88, P>0.05] at the end of 1 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 did not show any significant release profile at the end of 1 h study in both 2 % and 4 % RCCM compared to control medium. The similar trend was observed in 3 h [F (2, 15) = 0.63, P>0.05], 6 h [F (2, 15) = 0.61, P>0.05]. Further, statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (2, 15) = 93.4, P<0.05] at the end of 9 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was no significant change in release profile for CTDC-5 formulation in between 2% and 4% w/v RCCM. The similar trend was observed in 12 h [F (2, 15) = 70.3, P<0.05] study. Furthermore, statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (2, 15) = 79.2, P<0.05] at the end of 15 h study for CTDC-5 formulation. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 formulation showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was significant increased release profile for CTDC-5 formulation in 4 % w/v RCCM compared to 2 % RCCM. The similar trend was observed in 18 h [F (2, 15) = 134.4, P<0.05] and 21 h [F (2, 15) = 95.3, P<0.05] study.

Fig-4 illustrates the percent cumulative *in-vitro* drug released profile of 5-FU from CTDC-5 formulation containing 30% guar gum with rat caecal content (2 & 4% w/v) and without rat caecal content (Control). Statistical analysis by One way ANOVA revealed that there was insignificant difference among groups [F (2, 15) = 0.61, P>0.05] at the end of 1 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 did not show any significant release profile at the end of 1 h study in both 2 % and 4 % RCCM compared to control medium. The similar trend was observed in 3 h [F (2, 15) = 0.66, P>0.05]. Further, statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (2, 15) = 74.4, P<0.05] at the end of 6 h study for CTDC-5. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was no significant change in release profile for CTDC-5 formulation in between 2% and 4% w/v RCCM. The similar trend was observed in 9 h [F (2, 15) = 91.3, P<0.05], 12 h [F (2, 15) = 103.6, P<0.05] Furthermore, statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (2, 15) = 122.3, P<0.05] at the end of 15 h study for CTDC-5 formulation. *Post hoc* analysis by Student Newmann keuls test revealed that CTDC-5 formulation showed significant increased profile both in 2% and 4% w/v RCCM compared to control medium. However, there was significant increased release profile for CTDC-5 formulation in 4 % w/v RCCM compared

to 2 % RCCM. The similar trend was observed in 15 h [F (2, 15) = 134.2, P<0.05], 18 h [F (2, 15) = 134.4, P<0.05] and 21 h [F (2, 15) = 95.3, P<0.05] study.

In the present DSC study a 1:1 ratio of drug and excepients was used to maximize the possible occurrence of interactions of interaction would take place. Incompatibility or interaction occurs during DSC when there is (i) change in peaks onset, peak maximum or peak recovery (ii) disappearenc of endothermic peaks and/ or (iii) appearance of new peaks. During testing the thermograms of individual excipients, drug and powder mixture of drug and excipients was obtained in order to predict the possibilities of compatibility or interactions between them. Thermograms of Fig. 5 show the various possible combinations of drug with excipients melting endotherms. Scan-A was thermogram of 5-FU, which shows a peak onset at 281.3°C, peak maximum at 286.6°C and occurring of peak recovery at 293.4°C. Then further abrasion or noises appeared after melting point of drug demonstrating its decomposition with no further heating. The melting endotherms of Scan-B, C and D showed slight early peak maximum at 283.2°C; 289.9°C and 282.2°C respectively while Scan-E demonstrating an equivalent peak maximum at 286.3°C and Scan-F exhibited peak slightly post shifted to 290.6°C. Some changes in peak height and area were expected due to reasons of possible differences of geometric mixing ratios in combination may be considered its compatibility. Also the endotherm peak height and enthalpy (ΔH) were found decreased but may be considered as formulation excipients of dosage form for colon delivery.

Fig-6 revealed the cross sections of cleaved surface through coated capsules with HPMC: EdS-100 (2:4) as coating ratios. The contours of the enteric coating film of EdS-100 are seen to adhere with the irregular surface of the HPMC pre-coating material. Further, Fig-7 showed that the high strength of bond between HPMC and EdL-100 film is a combination of the irregular surface and the tackiness of the partially dissolved surface to give a uniform coating configurations of 4:3 ratios. It was observed that the strength of interface was superior to that of either substrate or the coating material of Eudragit®L/S-100 polymers. No pores or cracks were observed due to well-controlled coating process ensuring gastric integrity and compatibility between HPMC and anionic methacrylate dispersions.

Stability studies were carried out at 40ºC/ 75% RH for 6 month periods to access their long term stability. The formulation was subjected to *in-vitro* percent drug release study in simulated gastro-intestinal fluids of stomach, small intestine and colon showed no remarkable differences in the release pattern as compared to same formulation before storage at 40ºC/ 75% RH for 6 month. Also, no changes in physical appearances and residual drug content were noted for the 5-FU containing selective formulations. The result attributed to long-term stability of about 2 years and its potential market utility.

DISCUSSION

The *In-Vitro* studies indicated that the optimized formulation CTDC-5 with EdS-100 containing 30% of guar gum were capable of protecting the drug release in upper GI tracts, whereas they improved drug release in simulated colonic fluids containing rat caecal contents. Further, during storage at 40ºC/75% relative humidity for 6 months showed no significant alterations in drug

release profile and its physical appearance. Furthermore, there was no interaction between drug and polymer used in the formulation and SEM report supported towards good release profile.

The proposed formulation was based on combined pH-dependent and microbially triggered approach on modifications to Pulsatile Release Technology (Pulsincap™) for achieving colon specific release [42-44]. The formulations consist of water insoluble hard gelatin capsule body (combined treatment of formaldehyde vapor and heat exposure) filled with guar gum matrix containing drug contents were covered by a water-soluble cap. Then the whole system was coated with hydrophilic swellable HPMC (inner layer) and an enteric coating layer of Eudragit S-100 (outer layer) to avoid the gastric emptying variables. The enteric layers eroded when capsule enters the higher pH region of dissolution fluids. In contrast to gelatin, HPMC has a rough surface, which provides good adhesion to the coating and increases the water permeability to reduce the lag time (3-5 h). When capsule enters the small intestinal conditions (about pH 7.0) the enteric coating gets eroded and guar gum matrix was so adjusted to sustain the drug release during the lag time of 3-5 h, thereafter complete release only in the colonic region.

On coating the capsular body by gastroresistant film over the swellable hydrophilic layer helps to overcome the problem related to gastric pH and emptying time variability [45]. Enteric coating of formulations is able to prevent the rapid swelling and/ or disintegration of the polysaccharides matrix (pH-independent) during its transit through the upper hostile GI tracts. The enteric layers eroded when capsule enters the higher pH region of dissolution fluids (≥ pH 7.0). In contrast to gelatin, HPMC is often used as a pre-coating material for enteric-coated formulations having a rough surface, which provides good polymer-to-polymer adhesion and increase the water permeability [46]. The swellable guar gum plug upon water penetration expands slowly and induced for erosion of outer barrier coatings. The drug is released from the inner reservoir plug containing guar gum after a certain lag time of 3-5 h only in the colonic region.

Drugs, which are used for the treatment of diseases associated with colon, require passage of formulation in intact form through stomach and small intestine and release of whole amount of drug in colon [47]. The conventional dosage form normally dissolves in the stomach and small intestine and drug absorbs from these regions of the GI tract and a very less amount of drug reaches up to colon. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target site in the optimum amount for right period of time, thereby causing little toxicity and minimal side effects. Formaldehyde vapor treatment modifies the solubility property of gelatin in biological fluids. The vapor treatment favors cross-linking between amino groups in the gelatin molecular chain with aldehyde group due to Schiff's base condensation, which may result into unpredictable decreased solubility of capsule shells [48, 49]. The untreated capsules disintegrated and solubilized within 20 min while formalin treated capsule body remained intact for more than 24 h during disintegration testing. The results revealed that on combined exposure to formaldehyde vapor and heat treatment at 50°C for 30 min was found satisfactory to prevent the disintegration of capsule body for about 24 h indicating its suitability for colon targeting. Drug content studies were carried out to ensure uniform distribution of drug in the formulation. The results revealed that the percent drug content found between 95-101% was within I.P.1996 limits of content variation (±15% average).

An attempt had been made to achieve colon specific delivery using guar gum polysaccharides. Krishnaiah et al. [50] have investigated guar gum based matrix tablets of mebendazole where as Momin et al. [51] have proposed guar gum based formulations for the colon targeted delivery of sennosides. The developed system consisting of matrix guar gum containing hard gelatin capsule coated with enteric polymers has combined pH sensitive property and biodegradability in the colon. As far as the treatment of colonic diseases is concerned, it is of utmost important to ensure the delivery of drug in intact form in the vicinity of target organ. The condition of release of maximum amount of drug in upper part of GI tract necessiciated for manipulation in the formulation to control the drug release as per desired. The formulations were coated with inner coating of HPMC and outer coating with EdL/ EdS-100 in different ratios to retard the release of drug until pH reaches above 6.0. EdL-100 and EdS-100 are the co-polymers of methacrylic acid and methyl methacrylate. The ratio of carboxyl to ester group is approx. 1:1 in EdL-100 and 1:2 in EdS-100. These polymers contain ioniazable carboxyl group and dissolves as the pH moves towards alkaline range, due to formation of salt [52]. HPMC is often used as pre-coating material for enteric-coated capsule formulation would result into “good polymer to polymer adhesion” and compatibility and additionally improves hydrophilicity to the formulation [53]. Gelatin capsules have a very glossy surface due the fact that the amount of regular reflection from the surface is high and the amount of diffuse reflection is low. As on applying coating film of EdL/ EdS-100 film over gelatin capsules often suffer from insufficient adhesion between the shells and the coatings. Thus previous workers in the area of enteric coating have found it necessary to pre-coat gelatin capsules with a cellulose derivative to promote adhesion of polymers to the capsule shell [54, 55]. Pre-coating with HPMC polymeric film provided matte like surface along with more irregular surface to improve adhesion and stability.

In order to evaluate the susceptibility of guar gum polymer to undergo enzymatic biodegradability action by colonic microfloras, the *in-vitro* drug release studies were also performed in presence of rat caecal content in simulated colonic fluid at pH 6.8. The developed colon targeting delivery systems successfully retarded the drug release until it enters into the distal small intestine or up to colon as after 5 hr of previous release testing not more than 10% (approx.) of 5-FU was released. The protective coating of the enteric polymer is completely removed/ dissolved due to high alkaline pH range of small intestinal fluids and the drug matrix system bearing guar gum is confronted with the colonic fluids. Guar gum is a natural polysaccharide that shows susceptibility to colonic enzymes and hence to investigate the polymeric biodegradability to colonic enzymes, *in-vitro* studies were carried out in presence of rat caecal content. Conventional *in-vitro* drug release studies were carried out with 900 ml of dissolution medium. However, for present study it requires huge quantity of caecal matter, which seems to be practically inconvenient and uneconomical and hence *in-vitro* drug release studies were performed in 250 ml beaker containing 200 ml caecal content containing dissolution media was kept immersed in 900 ml vessel, as water bath of the apparatus. A remarkable improved cumulative percent drug release was observed for formulation in presence of rat caecal content at the end of 24 hr study when compared to control in simulated colonic fluid (pH 6.8) at different time intervals.

The release media containing rat caecal content was used to simulate the conditions prevailing in the colon and so far it it quiet evident from the testing results that guar gum matrix is susceptible to colonic enzymes released from caecal content. The release of drug from matrix is controlled

by the existence of hydration and erosion of guar gum polymer from the capsule's open end. The presence of rat caecal content in the dissolution medium secrets enzymes, which are responsible for degradation of diffused out layer of guar gum matrix and consequently drug release, is facilitated. Release of drug from the formulation depends on the relative strength of hydration/ eroded guar gum layer and activity of the colonic enzymes contents. The observation data shows that there was no significant increase in drug release even on increasing the concentration level of caecal contents from 2% w/v to 4% w/v in the dissolution media, which could be due to insufficient amount of enzyme level responsible for digestion of guar gum. Due to this reason only a maximum of up to 54 and 62% (approx.) drug was released after 21 hr with 2% w/v and 4% w/v rat caecal content respectively. Yet there are still drug entrapped to be release from the dosage forms. It is true that the amount of caecal matter in human beings, to which dosage form is supposed to be exposed on oral administration, is manifold greater than that used in the present *in-vitro* experimental conditions (4 g/ 100 ml [56]). In an experiment Vanden Mooter et al. [57] might have used 20 g/ 200 ml of rat caecal content to study the enzymatic degradation of azo polymers designed for colon specific drug delivery due to these reasons. As on using such a high level of caecal contents for routine *in-vitro* experiments are inconvenient and uneconomical, therefore an attempt was made to induce enzymes, which specifically act on guar gum, so that it can be carried out with lower level of caecal matter in the dissolution media. Hence in the present study it is required to increase the enzymatic activity by carrying out *in-vitro* release in presence of 2% w/v and 4% w/v rat caecal content obtained after 2, 4 and 6 days of enzyme induction. A set of rats were treated orally with 1 ml of 2% w/v aqueous dispersion of guar gum for successive 2, 4 and 6 days to induce the enzyme that specifically act on guar gum during the passage of formulation through the colon.

The results of drug release in presence of 2% w/v and 4% w/v caecal matter obtained after 21 hr of testing, the total percent drug released at 2% w/v level was significantly higher than those released without induction. The results also indicated that at different time interval the percent drug released was improved due to enzyme induction. The release of drug was found much faster during 18-21 hr testing period which may be due to the fact that during 18-21 hr the gel network was somewhat loosen along with enzymatic activity facilitated the release of drug. *In-vitro* studies after 2 days on enzyme induction with 2% w/v and 4% w/v rat caecal content shows prominent improved drug release in comparison to the testing performed without enzymatic induction. However, a complete release of drug from dosage form did not occur on increased level of enzymes and hence the situation demanded to increase the enzymatic induction periods from 2 to 4 days. The pre-treatment of animals with 1 ml of 2% w/v of guar gum dispersion was carried out for successive 3 days and the above experiment were repeated with 2% w/v and 4% w/v of caecal content in order to find out its influence on drug release rate. Induction of enzymes for the period of 4 days exhibited a pronounced influence on the release rate of drug of all the formulations. It was found that there was significant ($p<0.05$) change slightly in drug release containing rat caecal content after 4 days of enzyme induction when compared to 2 days induction period. However, an increased drug release effect in dissolution media containing 2% w/v caecal matter during 21 h of testing was obtained after 4 days. At 15 hr, the capsules were found to be broken/ disintegrated into 4 to 5 piece and thereby increasing the specific surface area available to the dissolution fluid and enzymatic actions. Hence, there was a steep rise in % drug release was noted between 15 and 18 hr of the testing. The increased surface area to be exposed also resulted into enhanced release rate during the periods. Furthermore, the release of

drug was significantly increased with 2% w/v and 4% w/v caecal content when compared to study involving no caecal content. The hydrated gel network was not very firm due to this reasons the erosion and digestion by colonic enzymes was higher and consequent a higher release of drug during 15-18 hr release rate study period.

Even though there was an improvement in percent drug release rate obtained after 4 days of treatment with 4% w/v caecal matter but there was still some drug remained entrapped in the formulations need further release. Hence again enzyme induction period was increased to 6 days and further *in-vitro* experiments was repeated with 2% w/v and 4% w/v rat caecal content in order to find out its influence on release rate from dosage form. Enzymatic activity was further improved after 6 days of enzyme induction and that is evident from the release of higher percent of drug in comparison to previous 4 days treatment studies. Thus there was a marked improvement in total percent drug release was noted after 5 days of induction when compared with those without induction. A rise in percent drug release was observed from 9 hr onwards with 2% w/v caecal content which may be due to induction treatment for 6 days. But, a steep rise was seen in percent drug release from 12 hr itself with 4% w/v rat caecal content, which may be due to high level of caecal enzymes induced by pre-treatment of rats for 6 days causing disintegration/ breaking of capsules resulted into fast erosion of hydrated gum. The results clearly demonstrated the susceptibility of guar gum to enzymatic action of rat caecal content as percent drug release in presence of different level of caecal content was better than containing no caecal content in release medium.

Fig. 5: DSC thermogram obtained from (A) 5-FU (B) 5-FU + guar gum (c) 5-florouracil + guar gum + lactose (D) 5-FU+ guar gum + lactose + HPMC (E) 5-FU + guar gum + lactose + HPMC + Eudragit®L-100 (F) 5-FU + guar gum + lactose + HPMC + Eudragit®S-100.

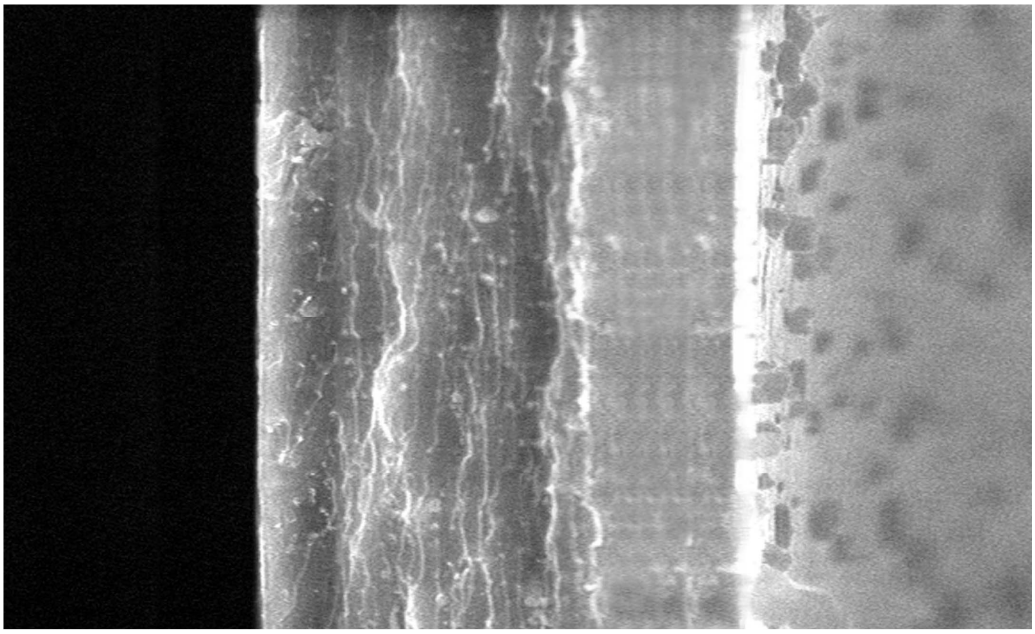


Fig. 6: Scanning electron micrograph (SEM) showing cross section of cleaved coated hard gelatin capsule with HPMC: Eudragit®S-100 (2:4) ratio.

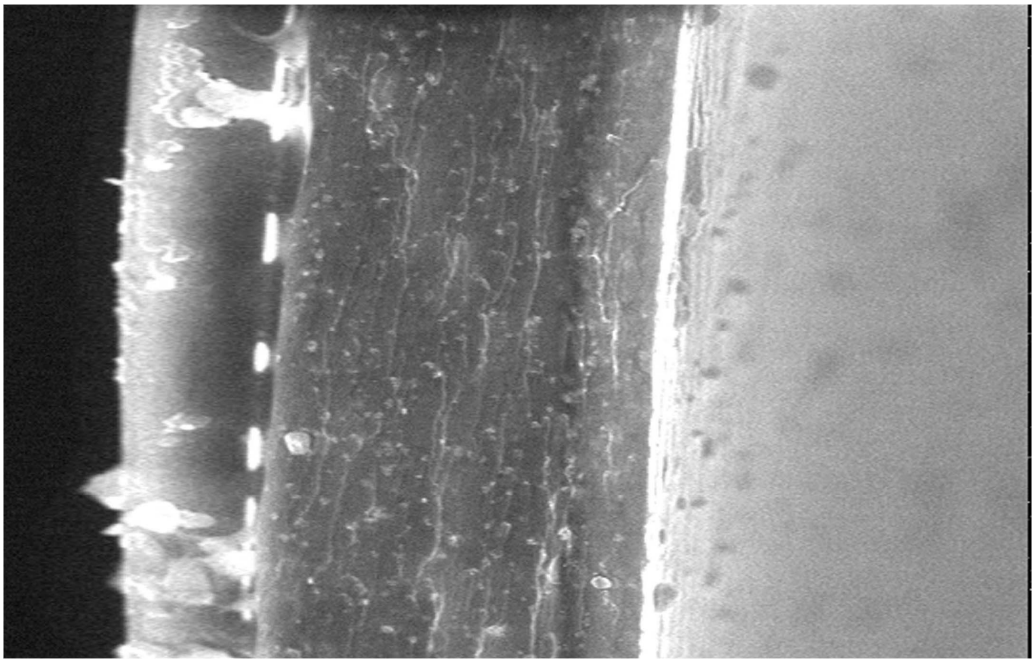


Fig. 7: Scanning electron micrograph (SEM) showing cross section of cleaved coated hard gelatin capsule with HPMC: Eudragit®L-100 (4:3) ratio.

CONCLUSIONS

Thus the results obtained after 2 and 4 days pre-treatment with caecal content indicated that the enzymes metabolizing guar gum can be a good inducer to improve the release profile. Furthermore, pre-treatment with guar gum for 6 days improved the enzyme induction as the drug released rate-increased up to 97% (approx.) at 4% w/v level of caecal content. Hence, it can be concluded that, the presence of 4% w/v rat caecal content in the dissolution medium obtained after 6 days of enzyme induction is best suitable condition for *in-vitro* evaluation of formulation containing matrix guar gum coated with combined HPMC and enteric Eudragit®S-100 polymer (2:4 ratios) for colon specific targeting.

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REFERENCES

[1] P Watts; L Illum. Colonic drug delivery. *Drug Dev Ind Pharm,* **1997**, 23, 893-913.
[2] K Ikesue, *et al*. *Control Rel Bioact Mater,* **1991**, 18, 580-581.

[3] VHL Lee. Changing needs in drug delivery. In: Peptide & protein drug delivery. Lee VHL edn. New York: Marcel Dekker, **1991**, 1-56.
[4] T Bussemer, *et al*. Pulsatile drug-delivery systems. *Crit Rev Ther Drug Carrier Syst*, **2001**, 18, 433-458.
[5] RJ Mrsyn. *J Control Release*, **1992**, 22(1), 15-34.
[6] M Ashford, *et al*. *J Control Release*, **1994**, 30, 225-232.
[7] DR Friend. *Adv Drug Deliv Rev*, **1991**, 7, 149-199.
[8] A Rubinstein. *Crit Rev Ther Drug Carr Syst*, **1995**, 12(2-3), 101-149.
[9] JT Fell. *J Anatomy*, **1996**, 189, 517-519.
[10] A Gazzaniga, *et al*. *Int J Pharm*, **1994**, 108, 77-83.
[11] VR Sinha, R Kumaria. *Acta Pharmaceutica*, **2003**, 53, 41-47.
[12] R Peters, R Kinget. *Int J Pharm*, **1993**, 94, 125-134.
[13] S Milojavic, *et al*. *Pharma Sci*, **1995**, 5, 77-82.
[14] A Rubinstein, *et al*. *Int J Pharm*, **1992**, 84, 141-150.
[15] DR Friend. Glycosides in colonic drug delivery. In: Friend DR edn. Oral Colon-Specific Drug Delivery. London: CRC Press, **1992**, 153-187.
[16] K Niwa, *et al*. *J Drug Target*, **1995**, 3, 83-89.
[17] T Takaya, *et al*. *J Pharm Pharmacol*, **1995**, 47, 474-478.
[18] R Kinget, *et al*. *J Drug Target*, **1998**, 6, 129-149.
[19] M Ashford, *et al*. *Int J Pharm*, **1993**, 95, 193-199.
[20] SS Davis, *et al*. *J Pharm Pharmcol*, **1984**, 36, 740-742.
[21] E Fukui, *et al*. *J Control Release*, **2001**, 70, 97-107.
[22] AM Goldstein, *et al*. Guargum. In: Whistler, R.L. edn. *Industrial gums, Polysaccharides & their detrivatives*. New York: Academic Press, **1993**, 303-331.
[23] MK Chourasia, SK Jain. *J Pharm Pharm Sci*, **2003**, 6, 33-66.
[24] SK Lanjhiyana, *et al*. *Indian Drugs*, **2007**, 44(3), 180-184.
[25] SL Gorbach. *Gastroenterol*, **1971**, 60, 1110-1129.
[26] GT Macfarlane, JH Cummings. The colonic flora, fermentation and large bowel digestive function. In: Philips SF, Pemberton JH, Shoter RG. edn. *The large intestine: Physiology, Pathophysiology and Diseases*. New York: Raven Press, **1991**, 51-92.
[27] AA Salayers, JAZ Leedle. Carbohydrates metabolism in the human colon. In: Hentges, D. J. (Ed.). *Human intestinal microfloras in health and disease*. London: Academic Press, **1983**, 129.
[28] L Yang, et al. *Int J Pharm*, **2002**, 235, 1-15.
[29] HN Englyst, *et al. FEMS Microbiol Ecol*, **1987**, 45, 163-171.
[30] MC Gohel, GM Sumitra. *J Control Release*, **2002**, 79, 157-164.
[31] SK Bajpai, *et al*. *J Appl Polymer Sci*, **2003**, 89(8), 2277-2282.
[32] M Sripathy, *et al*. *Eastern Pharmacist*, **1999**, 42, 97-98.
[33] MK Chourasia, SK Jain. *J Drug Target*, **2004**, 12(7), 435-442.
[34] YJ Jung, *et al*. *J Pharm Sci*, **2000**, 89, 594–602.
[35] AG Renwick. First-pass metabolism within the lumen of the gastrointestinal tract. In: Gorge, C.F., Shand, G., Renwick, A.G. edn. *Clinical Pharmacology and Therapeutics, Presystemic Drug Elimination*, London: Butterworth Scientific, **1982**, 1, 3–28.
[36] H Tozaki, *et al*. *J Pharm Sci*, **2001**, 90, 89–97.
[37] YV Rama Prasad, *et al*. *J Control Release*, **1998**, 51, 281-287.
[38] MK Chourasia, SK Jain. *J Pharm Pharm Sci*, **2003**, 6, 33-66.
[39] YSR Krishnaiah, *et al*. *J Control Release*, **2002**, 81, 45-56.

[40] VR Sinha, *et al*. *Int J Pharm*, **2004**, 269, 101-108.
[41] BR Mathews. *Drug Dev Ind Pharm*, **1999**, 25, 831-856.
[42] A Gazzaniga, *et al. STP Pharma Sci*, **1995**, 5(1), 83-88.
[43] ME McNeill, *et al.* Dispensing device. WO Patent 90/09168. **1990**; August 29.
[44] IR Wilding, *et al*. *Pharm Res*, **1992**, 9, 654-657.
[45] ME Sangalli, *et al. J Control Release*, **2001**, 73, 103-110.
[46] ET Cole, *et al*. *Int J Pharm*, **2002**, 231, 83-95.
[47] DR Friend. *Adv Drug Deliv Rev*, **1991**, 7, 149-199.
[48] VS Mastiholimath, *et al. Int J Pharm*, **2007**, 328, 49-56.
[49] GA Digenis, *et al*. *J Pharm Sci*, **1994**, 83, 915-921.
[50] YSR Krishnaiah, *et al*. *J Control Release*, **2001**, 77, 87–95.
[51] M Momin, K Pundarikakshudu. *J Pharm Pharma Sci*, **2004**, 7(3), 325-331.
[52] M Asford, *et al*. *Int J Pharm*, **1993**, 91, 241-245.
[53] E Cole, *et al*. *Int J Pharm*, **2002**, 231, 83-95.
[54] KS Murthy, *et al. Pharm Technol*, **1986**, 10, 36-46.
[55] H Kibbe. Crosspovidone. In: *Handbook of Pharmaceutical Excipients*, ed. Kibbe, H., United Kingdom: Washington, American Pharmaceutical Association. **2000**, 163-164.
[56] YV Rama Prasad, *et al. J Control Release*, **1998**, 51, 281-287.
[57]G Vanden Mooter, et al. *J Pharm Sci*, **1997**, 86, 1321-1327.