Research Article

IN-VITRO AND *IN-VIVO* RELEASE STUDIES OF METHOTREXATE FROM NOVEL ENTERIC COATED TIME-DEPENDENT MICROBIAL-TRIGGERED DRUG DELIVERY SYSTEMS FOR COLON SPECIFIC

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ABSTRACT

The present experiment is hypothesized to develop a single unit colon targeting delivery formulations for oral administration of methotrexate (MTX) as model drug based for effective treatment of colorectal cancer. Each batch of the capsules were coated with different thickness ratios of HPMC: Ed®S-100 (2:4, 4:2, 3:4, 4:3) into formulations F1(2:4), F2(4:2), F3(3:4) and F4(4:3) colon targeted drug capsule (CTDC) respectively. The *in-vitro* study revealed that only F1(2:4)CTDC and F3(3:4)CTDC formulations showed significantly increased gastro-resistance for 3 h at pH 7.4 compared to formulation F2(4:2)CTDC and F4(4:3)CTDC respectively. Further, *in-vitro* release data demonstrated that both the formulations F1(2:4)CTDC and F3(3:4)CTDC released significant amount of MTX in simulated colonic fluids (pH 6.8) containing 2 & 4% w/v rat caecal content at the end of 24 h studies compared to medium without caecal content. *In-vivo* gamma-scintigraphy revealed the colonic arrival and release profile of F1(2:4)CTDC more precisely. Furthermore, accelerated stability studies of F1(2:4)CTDC revealed absence of any interactions between drug and excipient used in the formulation. Therefore, the developed system could be a promising device to achieve greater site specificity, reduced side effects, cut down the conventional dose size and effective treatment for colon cancer disease.

Keywords: Colonic specific drug delivery; Guar gum polysaccharide; pH-sensitive polymers; Microbial degradations, *In-vitro* drug release.

1. INTRODUCTION

Targeted drug delivery system to the field of pharmacotherapy is gaining more importance during the last decade. It has been reported that the colon is beneficial for local treatment of number of pathological disorders such as colorectal cancer, chroh's disease, inflammatory bowel disease and amoebiasis [1]. It has been suggested that the colon does not possess the ideal anatomical & physiological features compared to upper gastrointestinal (GI) tract; however, it is the site having negligible brush-border membrane peptidase activity, longer retention time (20-30 h), high responsiveness to poorly absorbed drugs and perhaps less hostile recognized environments [2]. It is evident that the conventional dosage forms are delivering inadequate amount of drug to colon due to the absorption or degradation in the hostile upper GI tract. Further, the colon is suitable for targeting because of less acidic or enzymatic activity and invariable neutral pH [3]. Therefore, the colon would be a promising site for both local and systemic drug delivery system.

Literature review suggests that there are various approaches which have been proposed for oral delivery of drug(s) in order to achieve colon specific drug delivery system [4] that include time dependent delivery system [5], pH sensitive polymer coatings [6], microbially triggered enzymatic degradation by colonic bacteria [7], prodrug approach based delivery [8] and pressure controlled release systems [9].

It has been realized that the pH of GI tract progressively increases as we move from stomach to colon (pH 2- 8). On the contrary, the recent studies reported that the pH varies and declines significantly from the ileum to colon [10]. It is reported that pH-dependent target system showed poor site specificity due to large pH variations and transit time of GI tracts [11,12]. Further, it has been well documented that the release profile of the coated formulations is protected in the stomach and proximal part of small intestine, however, showing little site specificity at the distal part of small intestine [13].

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 [14]. The polysaccharide is hydrophilic in nature, which swells to form viscous gel like mass on

absorption of dissolution fluids or GI fluids. Further, it is well suggested that guar gum reduces the release profile in the upper GI tracts and is highly susceptible to degradation by the colonic microfloral environments. The hydration and viscosity of it is not affected in the dissolution medium over a wide range of pH [15]. Our previous study revealed that the formulation containing 30% guar gum is best suitable for colon targeting than 10%, 20% or 40% concentration of guar gum [16].

It has been well documented that the colonic micro floras are considered as triggering component for colon site specific delivery system. The colon consist of more than 400 bacterial species having population of 10^{11}-10^{12} CFU/ ml namely Bacteriodes, Eubacterium, Lactobacillus, Bifidobacterium etc. [17], those are responsible for fermentation and degradation of plant polysaccharides [18]. The responsible enzymes triggering the polymer degradation include β-xylosidase, β-D-glucosidase, β-D-galactosidase β-D-fucosidase [19]. Furthermore, it has been well established that the caecal content of rodent are utilizing more commonly as an alternative dissolution medium to overcome certain limitations of conventional USP dissolution testing for evaluating the colon specific delivery systems. Due to similarity with human colonic microfloras and fermentation of polysaccharides, the approach could avoid the limitations during designing of time and pH-dependent delivery systems. Methotrexate is chemically N-[-4-{[(2, 4-diamino-6-pteridinyl)-methyl] methylamine} benzyl] glutamic acid, used as a specific cell cycle inhibitor in the management of cancer.

Therefore, in the present experiment it is hypothesized to develop a colon targeted time-dependent pulsatile release delivery system for methotrexate following inner coating by HPMC and outer coating by Ed®S-100. Further, an in-vitro and in-vivo correlation is established.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Methotrexate (MTX) was generous gift from M/s. Unimed Technology Pvt. Ltd. Gujarat (India). Eudragit®S-100 (Ed®S-100) was donated by Rohm Pharma, Darmstadt (Germany) & 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 & Hard gelatin capsule sizes#2 were obtained from Sunil Health Care Ltd., Rajasthan (India). Diethylene triamine penta acetic acid (DTPA) was obtained from Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal (India). All other reagents & organic solvents used were of analytical/ pharmacopoeia grade, and purchased from commercial suppliers.

2.2 Preparations & Coating of Colon Targeting Delivery Capsule (CTDC)

The formulation matrix in all cases was consisted of 20 mg drug with 30% guar gum, was filled into hard gelatin capsule (size #2) and rest of volume was adjusted by inert lactose. The joint of the capsule was sealed with a small amount of 5% ethyl cellulose solution. Each batch of the capsules was coated for inner coating by HPMC (hydrophilic layer) and outer coating by Ed®S-100 (enteric layer), by dip coating method into the polymeric solution of HPMC and Ed®S-100 to ensure the formation of a uniform and thin covering over the capsule. In order to enhance the elasticity of Ed®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**:** Ed®S-100 (2:4, 4:2, 3:4, 4:3) into formulations F1(2:4)CTDC, F2(4:2)CTDC, F3(3:4)CTDC & F4(4:3)CTDC respectively by dipping twice, thrice & four times respectively in each coating solution at room temperature. The film was allowed to dry with the help of dryer with an inlet temperature of 35-40ºC.

2.3 Determination of Drug Content

The formulation was crushed and dissolved in phosphate buffer saline (PBS) solution of 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 methanolic PBS (pH 7.4) solution and filtered through Whatman No.1 filter paper. The absorbance and percent drug content of the filtrate was recorded with the help of Double-Beam UV-Spectro-photometer. The test was performed with formulations by assaying them individually according to USP limits.

2.4 *In-vitro* Release Studies

Drug release studies were carried out to assess the ability of coats/ carrier to remain intact in the physiological pH conditions of stomach & intestine using USP Dissolution Rate Test Apparatus (Basket type, 100 rpm, 37±0.5ºC). The coated formulations were tested initially at pH 1.2 for the first 2 h in simulated gastric fluids (SGF) containing 0.1 N Hydrochloric acids (750 ml) as the average gastric emptying time is about 2 h. Then the dissolution medium was added with 1.7g of KH_2PO_4 and 2.225g of $Na_2HPO_4.2H_2O$, adjusted to pH 7.4 using 1 M NaOH which were continued for another 3 h. At regular time intervals, 5 ml sample aliquots were withdrawn by replacing an equal volume of fresh medium to adjust the sink conditions & analysed for MTX at λmax of 256 nm using Double-Beam UV-Spectrophotometer in order to investigate whether the prepared formulations could restrict drug release in the adverse environment of gastric and intestinal environments.

2.5 Preparation of Rat Caecal Content Medium (RCCM)

Healthy adult Wistar rats aged 2-3 months of either sex weighing between 150-200 g were used for the experimental study. The rats were housed in colony cages under standard conditions (25ºC ± 1ºC, 12 h light-dark cycle & 45-55 relative humidity) were allowed freely access to their normal laboratory chow diet (16% proteins, 66% carbohydrates & 08% fats) & water *ad*

libitum. The Institutional Animal Ethics Committee of the University Department approved the experimental protocol under strict compliances of CPCSEA guidelines. Rats were sacrificed before 30 min of commencing drug release studies & the caecum was exteriorized for content collection. The caecal content (anaerobic nature) were immediately transferred into PBS (pH 7.4) to obtain an appropriate 2 % & 4% w/v concentration solution which was previously bubbled with nitrogen gas to maintain an anaerobic environment [20]. The obtained filtered caecal suspension was previously sonicated for 25 min at 4ºC in an ice bath to disrupt cell wall for releasing of bacterial populations and thereafter followed by centrifugation at 20000 rpm speed at 4ºC for 35 min in order to obtain clear caecal content medium.

2.5.1 *In-vitro* Release in Presence of Rat Caecal Content Medium (RCCM)

In-vitro drug release studies were performed in the presence of rat caecal contents to assess the susceptibility of guar gum affecting the performance of delivery systems triggered by colonic bacteria. The drug release studies were performed by using USP Dissolution Rate 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 supply was kept immersed in water bath for the dissolution test apparatus [21]. The formulation which was subjected previously to *in-vitro* release studies in SGF pH 1.2, 2 h & then at pH 7.4 for 3 h were immersed with caecal content in dissolution medium to give final dilutions of 2% w/v concentration. At different time intervals, 2 ml sample media was pippeted out regularly & compensated with freshly prepared PBS (pH 7.4) with same amount & the studies was continued till completion of 24 h. The withdrawn samples after volume made upto 10 ml was filtered through a 0.22 µm membrane filter was quantified at λmax of 256 nm using Double Beam 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.

2.6 *In-vivo* Gamma scintigraphy Study

Six healthy rabbits (aged 1 yr old, weighing between 800-1200 g of either sex) was divided into two groups of 3 animals each and fasted overnight for 10-12 h prior to commencement of experiment in order to standardize the conditions of GI motility. The study for nuclear imaging was performed at Dept. of Nuclear Medicines, Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal (India). Institutional Animal Ethics Committee approved the protocol. Each subject was administered the capsule formulation loaded with ^{99m}Tc-DTPA complex (tracer) followed by sufficient volume of water to outline the GI tract. The gamma camera had a field view of 40 cm was fitted with a medium energy parallel hole collimator and was set to detect 140 KeV gamma radiations emitted by ^{99m}Tc-DTPA were imaged. Subsequent anterior images for the movement of each labeled capsules from stomach to colon were recorded at every 1 h interval during the first 3 h and thereafter at 2 h for further 6 h by keeping in front of E-Cam Single Head gamma camera (Siemen's Germany). The image was recorded using online computer system (Macscnsetch, Germany) by linkage with gamma camera and stored in magnetic disk.

2.7 Stability Studies

Stability studies were conducted for the potential formulation F1(2:4)CTDC to access their long term stability [22]. The sample was stored at 40ºC/ 75% relative humidity 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% relative humidity for 6 month periods.

2.8 Differential Scanning Calorimetry:

DSC study was undertaken to detect any possible physical or chemical interaction takes place between drug & excipient which affects the compatibility & stability of the formulation by using differential scanning calorimeter (Dupont USA 900 Model). Samples (2-6 g) were placed in flat bottomed aluminum pans & hermetically sealed. The probes were heated from 25ºC to 600ºC at rate of 20ºC/ min under nitrogen atmosphere (50ºC/ min.). Thermograms of individual drug & drug-mixture were obtained and recorded properly.

2.9 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.

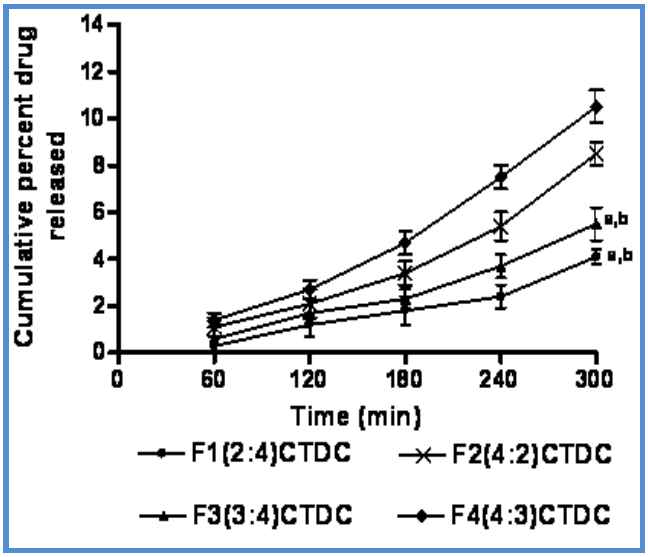
3. RESULTS

3.1 *In-Vitro* Drug Release in simulated gastric and small intestinal fluid

Fig-1 showed the drug release profile of MTX with different polymer coating ratios in simulated gastric (pH 1.2) and small intestinal (pH 7.4) fluids. Statistical analysis by One way ANOVA revealed that there was significant difference among groups [$F(3, 20) = 1.94$, $P>0.05$] at the end of 2 h study design. *Post hoc* analysis by Student Newmann keuls test revealed that the formulations F1(2:4)CTDC, F2(4:2)CTDC, F3(3:4)CTDC and F4 (4:3) CTDC were not significantly different from each other. Further, statistical analysis by One way ANOVA revealed that there was significant difference among groups [$F(3, 20) = 2.75$,

P<0.05] at the end of 5 h study design. *Post hoc* analysis by Student Newmann keuls test revealed that the formulations F1(2:4)CTDC and F3(3:4) CTDC showed significant decrease in drug release profile compared to both F2 (4:2) CTDC and F4(4:3)CTDC. However, there was no significant difference between F1(2:4) CTDC and F3(3:4)CTDC formulations indicating its potential to remain intact in simulated gastro-intestinal conditions at pH 1.2 & 7.4 respectively.

Figure 1: *In-vitro* drug release profile of MTX from F1(2:4)CTDC, F2(4:2)CTDC, F3(3:4)CTDC & F4(4:3)CTDC in simulated GI fluids at pH 1.2 and 7.4 for time intervals of 2 h and 3 h respectively. All the values are expressed in Mean ± SD. [a]P<0.05 compared to F2(4:2)CTDC and [b]P<0.05 compared to F4(4:3)CTDC (One-way ANOVA followed by Student Newmann keuls test).



3.2 *In-vitro* Drug Release Studies in Rat Caecal Content Medium

The percent cumulative *in-vitro* drug released profile of MTX from formulation F1(2:4) CTDC containing 30% guar gum with rat caecal content (2 & 4% w/v) and without rat caecal content (Control Study) is depicted in Fig-2 (A).

Statistical analysis by One way ANOVA revealed that there was insignificant difference among groups [F (2, 15) = 0.74, P>0.05] at the end of 1 h study for F1(2:4)CTDC formulation. *Post hoc* analysis by Student Newmann keuls test revealed that F1(2:4)CTDC formulation did not show significant release profile at the end of 1 h study both in 2 % and 4 % RCCM compared to control medium. Further, statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (2, 15) = 78.03, P<0.05] at the end of 3 h study for F1(2:4)CTDC formulation. *Post hoc* analysis by Student Newmann keuls test revealed that F1(2:4)CTDC formulation 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 F1(2:4)CTDC formulation in between 2% and 4% w/v RCCM. The similar trend was observed in 6 h [F (2, 15) = 75.48, P<0.05] and 9 h [F (2, 15) = 73.79, P<0.05] study. Furthermore, statistical analysis by One way ANOVA revealed that there was significant difference among groups [F (2, 15) = 79.47, P<0.05] at the end of 12 h study for F1(2:4)CTDC formulation. *Post hoc* analysis by Student Newmann keuls test revealed that F1(2:4)CTDC 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 F1(2:4)CTDC formulation in 4 % w/v RCCM compared to 2 % RCCM. The similar trend was observed in 15 h [F (2, 15) = 154.5, P<0.05], 18 h [F (2, 15) = 95.13, P<0.05] and 21 h [F (2, 15) = 112.5, P<0.05].

Figure 2: Percent cumulative *in-vitro* drug released profile of MTX from formulation F1 (2:4)CTDC (A) and F3(3:4)CTDC (B) containing 30% guar gum with (2 & 4% w/v) and without rat caecal content. All the values are expressed in Mean ± SD. [a]P<0.05 compared to control and [b]P<0.05 compared to 2% w/v RCCM (One-way ANOVA followed by Student Newmann keuls test).

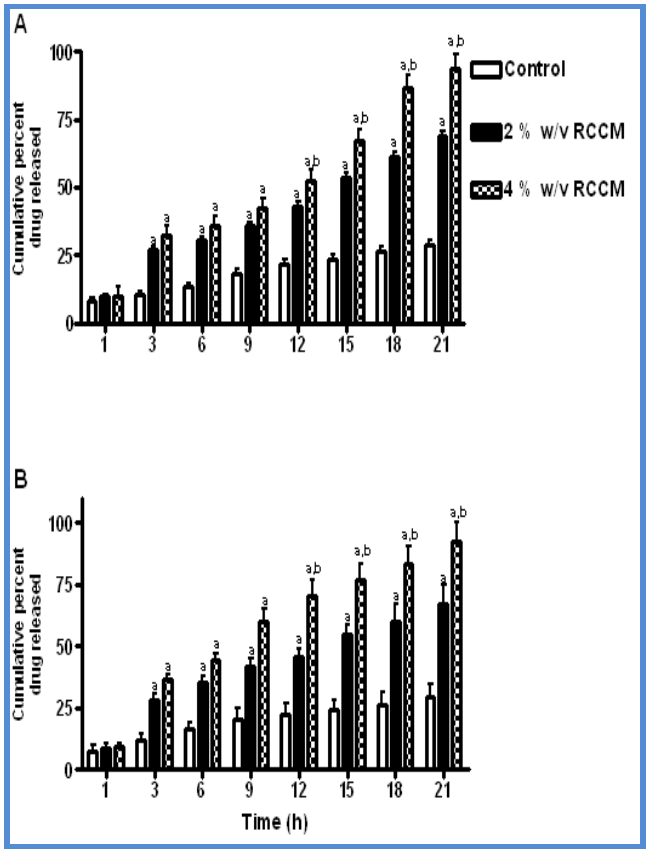


Fig-2 (B) revealed the percent cumulative *in-vitro* drug released profile of MTX from formulation F3(3:4)CTDC containing 30% guar gum with rat caecal content (2 & 4% w/v) and without rat caecal content (Control Study) in order to evaluate the susceptibility of guar gum polymer to undergo enzymatic action by colonic micro-floras. Similar trend was observed for F3(3:4)CTDC formulation at 1 h [F (2, 15) = 0.81, P>0.05], 3 h [F (2, 15) = 68.12, P<0.05], 6 h [F (2, 15) = 68.37, P<0.05], 9 h [F (2, 15) = 71.71, P<0.05], 12 h [F (2, 15) = 74.47, P<0.05], 15 h [F (2, 15) = 94.5, P<0.05], 18 h [F (2, 15) = 93.13, P<0.05] and 21 h [F (2, 15) = 95.5, P<0.05].

3.3 In-*vivo* Gamma scintigraphy

The *in-vivo* performance of the developed colon specific formulation by Gamma-Scintigraphy is depicted in Fig-3. The scintigraph as shown in Fig-3(A) clearly showed that a small amount of tracer was released in the stomach after 2 h interval. Scintigraph taken after 4 h revealed that somewhat more quantity of tracer was being released in small intestinal region Fig-3(B). After 4 h, the formulation enters into ascending colon and an increased release of tracer was found as shown in Fig-3(C). After 8 h the formulation was found remained in ascending colon but with distorted shape and liberated of higher amount of tracer considerably as shown in Fig-3(D). Further, Fig-3(E) showed a complete disintegration of the formulation at the end of 12 h. During 24 h the liberated radioactivity was distributed whole across the GI organs of ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon as depicted in Fig-3(F).

Figure 3: The *in-vivo* performance of the developed colon specific formulation F1(2:4)CTDC by gamma scintigraphy is depicted. Scintigraph shows gastro-intestinal transit and in-*vivo* release profile of F1(2:4)CTDC formulation at 2 h (A), 4 h (B), 6 h (C), 8 h (D), 12 h (E) and 24 h (F).

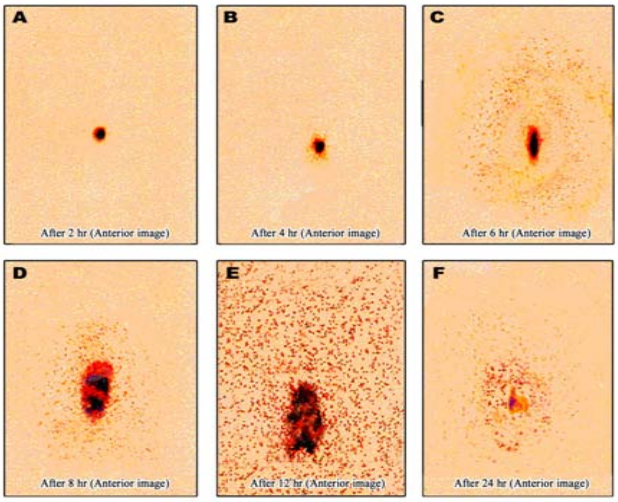


Table 1: Percent of MTX released from F1(2:4)CTDC in various simulated gastrointestinal fluids of 0.1 M HCl at pH 1.2 (2 h), pH 7.4 (1 h) & PBS at pH 6.8 containing 4% rat caecal content before and after storage at 45ºC/ 75% relative humidity for 6 months.

Time (h)	Dissolution medium (900 ml)	Percent of MTX released from F1(2:4)CTDC formulation	
		Before storage	After storage
0	1.2 pH	0 ± 0	0 ± 0
1		1.35 ± 0.05	1.72 ± 0.09
2		2.84 ± 0.09	2.61 ± 0.31
3	7.2 pH	5.46 ± 1.07	5.21 ± 1.01
6	6.8 pH PBS containing 4% w/v rat caecal content	19.82 ± 2.04	20.26 ± 1.84
9		32.71± 2.75	31.47 ± 3.08
12		40.38 ± 5.62	40.16 ± 4.76
15		52.72 ± 4.78	52.91 ± 4.42
18		61.35 ± 5.72	62.39 ± 4.44
21		68.56 ± 7.22	68.47 ± 5.72
24		94.26 ± 10.31	93.5 ± 11.62

All the values are expressed in Mean ± SD (Student-t test is performed at P<0.05).

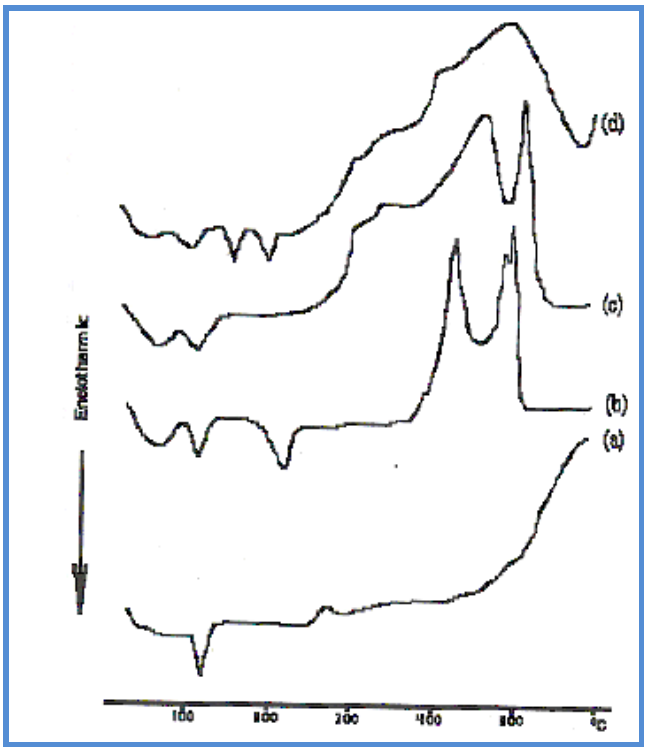
3.4 Stability Studies

The *in-vitro* percent drug release profile of F1(2:4)CTDC formulation in simulated gastro-intestinal fluids of stomach, small intestine and colon stored at 40ºC/ 75% RH for 6 months is depicted on Table-1. Statistical analysis by Student-t test revealed that the release profile of the formulation after storage conditions insignificantly different compared to before storage condition at 1 h [t (10) = 1.8, P<0.05], 2 h [t (10) = 1.7, P<0.05], 3 h [t (10) = 0.69, P<0.05], 6 h [t (10) = 0.39, P<0.05], 9 h [t (10) = 0.74, P<0.05], 12 h [t (10) = 0.79, P<0.05], 15 h [t (10) = 0.07, P<0.05], 18 h [t (10) = 0.14, P<0.05], 21 h [t (10) = 0.26, P<0.05] and 24 h [t (10) = 0.06, P<0.05]. Further, there were no significant changes in physical appearances and residual drug content for the MTX containing selective formulations.

3.5 Differential Scanning Calorimetric Study

The peaks of differential scanning calorimetric study of F1(2: 4)CTDC formulation is depicted in Fig-4. The sharp melting transition of pure MTX was observed at 110ºC of DSC thermogram. Drug-mixture with Ed®S-100, guar gum and HPMC shows endothermic peaks at 117ºC, 121ºC, 119ºC respectively indicating that there was absence of interaction or incompatibility between drug & other excipients of optimized formulations.

Figure 4: DSC thermograms of the formulation F1(2:4)CTDC containing (a) MTX; (b) MTX & Ed®S-100; (c) MTX, Ed®S-100 & Guar gum; (d) MTX, Ed®S-100, Guar gum & HPMC are depicted.



4. DISCUSSION

The *In-Vitro* and *In-Vivo* studies indicated that the formulation F1(2:4)CTDC 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. The guar gum were found susceptible by colonic microfloras by releasing about approximately 67-93% of MTX in simulated colonic fluids containing rat caecal contents at the end of 24 h under anaerobic atmosphere. 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 excipient used in the formulation.

The conventional dosage forms, which are used for colorectal cancer normally, dissolve & absorbs in the stomach & small intestine; thus a very less quantity of dose of drug reaches up to colonic region. The guar gum is able to protect the drug in the physiological environment of stomach & small intestine that was confirmed by dissolution studies containing SGF (pH 1.2) for 2 h & further in pH 6.5 for 3 h, mimicking lag time of 5 h[15] (Chourasia and Jain, 2003). The present study revealed that the formulations F1(2:4)CTDC and F3(3:4)CTDC released only up to approximately 4-10% of MTX after 5 h studies, which might be due to dissolution of surface drug particles being diffused out from capsule matrix. The coated guar gum matrix capsule was found to contain approximately 98-101% of MTX indicating for content uniformity, when subjected to drug content studies. The large deviation among formulations may be due to increased thickness of enteric Ed®S-100 layers as gastro-resistant in nature as compared to HPMC layers, which in turn promoted hydrophilicity on the coat, resulted into fast erosion. The lag time of 3-5 h prior to rapture of the capsule formulations was determined primarily by outer enteric coating of having mechanical erosion properties followed by water absorbing and swelling properties of inner coating layer. It has been observed from the released profile in medium with or without 2% and 4% w/v RCCM that both these F1(2:4)CTDC and F3(3:4)CTDC formulations were optimal, indicating less susceptibility of guar gum polymer to undergo enzymatic action by colonic microfloras may be the combined effects of diffusion and erosion. The study revealed the less susceptibility of guar gum to degrade by colonic bacteria may be responsible factor for drug release in physiological vicinity of colonic region.

The Gamma scintigraphy study showed that the susceptibility of guar gum polymer to colonic enzymes responsible for degradation and complete release of trace in the GI fluids. Although on entering into colon, the matrix capsules were found degraded by polysaccharides but the release and spreading of tracer was limited. However, there was marked improved in the intensity of tracer after complete disintegration of the formulation. Therefore it could be inferred that the HPMC:Ed®S-100 coated formulations can be successfully targeted to colon. Further, the stability studies at 40ºC/ 75% relative humidity for 6 month periods revealed that these formulations attributed to long-term stability of about 2 years and its potential market utility. The differential scanning calorimetry is suggesting that there was absence of interaction or incompatibility between drug & other excipients of optimized formulations.

Colon is considered as an important site for absorption and delivery of drugs used in the treatment of lower GI tracts diseases. The *In-Vitro* and *In-Vivo* correlation studies indicated that the formulation F1(2:4)CTDC containing 30% of guar gum was capable of protecting the drug release in upper GI tracts, whereas improved drug release in simulated colonic fluids containing rat caecal contents. Formulation developed were so designed to achieve delayed onset of drug release for a programmed lag time period (3-5 h) in the hostile environment of upper gastrointestinal tract. The guar gum were found susceptible by colonic microfloras by releasing about approximately 67-93% of MTX in simulated colonic fluids containing rat caecal contents at the end of 24 h under anaerobic atmosphere. During storage at 40ºC/75% relative humidity for 6 months showed no changes in drug release profile and its physical appearance. Further, there was absence of any interactions between drug and excipient used in the formulation. Hence, the study clearly indicated that the Ed®S-100 and HPMC coated capsules containing 30% guar gum a matrix drug carrier

may deliver the intact drug for local action into the colon for treatment of colorectal cancer.

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