Formulation And In Vitro, In Vivo Evaluation of Colon Targeted Drug Delivery System Of 5-Fluorouracil

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Abstract:
Colon-targeted drug delivery systems can provide therapeutic benefits including better patient compliance and lower costs. The present investigation is aimed to design a colon specific microbially triggered system using biodegradable co-polymer mixtures. The calibration curves of 5-FU were measured in distilled water, 0.1N HCl and phosphate buffer of pH 6.8 and 7.4 which showed good linearity. Compatibility study of pure drugs, excipients and their physical mixtures were evaluated and passed as per standards. Solubility determination was carried out in different solvents. Satisfactory results were found from evaluation of micromeritic parameters such as flow property, in-vitro dissolution study and kinetic study. The prime focus of the study was to design and evaluate a swelling dependent delayed release system for a colonic delivery of anticancer agent 5-Fluorouracil (5-FU) and further to determine the effects of carboxy polymer (Carbopol 71G-NF) on release behavior of 5-FU from a matrix tablet system containing different amounts of inulin (a biodegradable oligofructose) aiding in enzymatic degradation by colonic microflora. Mixed film coating with a blend of Ethyl cellulose: Eudragit®S-100 (2:1) at coat weight levels of 2%w/w, 4%w/w and 6.0%w/w was carried out respectively, which further retarded the drug release in the initial hours of the in-vitro dissolution profile. Swelling studies were also carried out on uncoated matrix tablet batches. The releases studies with or without rat cecal contents were performed on optimized batches and the samples were analyzed by a validated RP-HPLC method. In-vitro rat cecal study results revealed that complete drug release would occur from the tablets in the human colonic microenvironment. The study revealed an effective site-specific delivery of a hydrophilic chemotherapeutic agent, 5-FU to the colon for the treatment of various local as well as systemic pathologies.

Keywords: 5-FU, Colon, Targeted drug delivery system, RP-HPLC.

INTRODUCTION:

Colon targeted drug delivery systems have gained a great deal of attention as potential carriers for the treatment of colonic diseases with reduced systemic side effects and for the enhanced oral delivery of various therapeutics vulnerable to acidic and enzymatic degradation in the upper gastrointestinal tract. In recent years, the global pharmaceutical market for biologics has grown, and increasing demand for a more patient-friendly drug administration system highlights the importance of colonic drug delivery as a noninvasive delivery approach for molecules. Colon-targeted drug delivery systems can provide therapeutic benefits including better patient compliance and lower costs.

Natural polysaccharides are being extensively manipulated for the colon drug delivery systems. Nowadays, therapeutic compositions are being investigated that can effectively play a versatile role. Colon cancer is caused by a cascade of genetic mutations leading to progressively disordered local DNA replication and accelerated colonocyte replication. The progressive accumulation of multiple genetic mutations results in the transition from normal mucosa to benign adenoma to severe dysplasia to frank carcinoma (Capped, 2005). In brief, colon cancer develops in the colonic region of the lower gastrointestinal (GI) tract and usually develops slowly over a period of many years. 5-Fluorouracil (5-FU), a pyrimidine analog is one of the most extensively employed antineoplastic antimetabolite for colon cancer as well as breast cancer for six decades. It is sparingly water soluble (< 0.1 g/100 mL at 19 °C, logP ~ -0.8 and pKa ~ 8.02) drug which interferes with DNA synthesis by blocking the thymidylate synthetase conversion of deoxyuridylic acid to thymidylic acid. Effects on RNA occur especially with bolus administration. 5-FU is cell cycle phase specific (S-Phase) (www.drugbank.ca). 5-FU is well distributed into tumors, intestinal mucosa, bone marrow, liver and other tissues.

MATERIALS AND METHODS

Materials

5-Fluorouracil was obtained as gift sample from (5-FU) Manus Aktteva Biopharma PVt. LTD, Ahmedabad, Gujarat, India. HPMC was obtained as a gift sample from Kayel Medichem Private Limited, New Delhi, India.

Carbopol 71G-NF (granular grade) was obtained as gift sample from Neutron Drugs & Pharmaceuticals Private Limited, Hyderabad, Telangana, India. Xanthan gum was obtained as a gift sample from Kayel Medichem Private Limited, New Delhi, India.

METHODS

PREFORMULATION STUDIES ON 5-FLUOROURACIL (5-FU)

PHYSICOCHEMICAL CHARACTERIZATION OF 5-FU

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of the drug was performed on the FTIR spectrophotometer (60MHz Varian EM 360 Perkin Elmer) using KBr pellet technique.

Differential scanning calorimetry (DSC)

The characteristic endotherm and the enthalpy of the drug were obtained using DSC technique. Samples were placed in the A1 pans sealed using hydraulic press which were heated under the nitrogen flow of (20ml/min) at a scanning rate of 10°C from 20°C to 350°C. Empty aluminium pan was used as reference. The drug-excipient compatibility studies were also carried out in the physical mixtures of various tablet excipients and polymers ((in equal proportions 1:1) using DSC (Q20 series, TA instruments, USA).

In-vitro intestinal permeation studies

The permeability of the 5-FU was estimated *in-vitro* in the intestine (both small and large) of the pig using everted loop technique (Ruan et al., 2006). In this everted gut (or intestinal) loop model, a section of the pig intestine was taken from the slaughter house, flushed with buffer, and everted over a glass tube of similar diameter (3-4 cm). The intestine was at tightly tied other end with threads. The inlet tube was connected to the reservoir containing fresh phosphate buffer pH 6.8 solution kept at 37±0.5°C. Each inlet tube was filled with drug solution (1 mg/ml) and was placed in a glass container containing phosphate buffer (10 ml). The everted glass tubes connected with intestinal segments (both large and small intestine) were maintained at 37±0.5°C. 5 ml samples were collected at different intervals and replaced simultaneously by phosphate buffer pH 6.8 solution. The contents of the samples were subjected to UV spectrophotometric analysis at 266 nm. The rate of drug absorption was estimated in terms of flux. The experiment was performed in triplicate.

PREPARATION OF STANDARD PLOTS OF 5-FU USING UV-SPECTROPHOTOMETER

Preparation of standard plot of 5-FU in phosphate buffer (pH 6.8)

Similarly, stock solution of 5-FU having concentration 1 mg/ml was also prepared in phosphate buffer pH 6.8. To obtain phosphate buffer pH 6.8, 76.02 g of tribasic sodium orthophosphate was dissolved in 1L of distilled water and out of this 1L solution, 250 ml of solution was mixed in 750 ml of 0.1 N HCl and pH was adjusted to 6.8 which was used as dissolution medium. After vortex for 2-3 minutes, the stock solution was serially diluted in phosphate buffer (pH 6.8) at various concentrations from range 1-20 µg/ml. The solutions were measured for absorbance at λmax~274 nm using UV-visible spectrophotometer.

VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF 5-FU

The HPLC system (Shimadzu, Kyoto, Japan) consisting of LC-10AT pump, a SPD-10A UV-visible detector and a DGU-14A degasser model was used. The separations were carried out on a C-18 reversed phase column (Inertsil® ODS-3, 250x4.6 mm, 5µ). The column was operated at a temperature of 40°C. 50 mM KH2PO4 buffer (pH: 5.0) at a flow rate of 1.2 mL/min was used as the mobile phase. The wavelength of detection was 274 nm. The data acquired was processed by CLASS-VP® software (Shimadzu, Kyoto, Japan).

PREPARATION OF COLON TARGETED TABLETS OF 5-FU

Preparation of matrix tablets of 5-FU (Carbopol-inulin matrix-based system)

Matrix tablets (tablet weight-300 mg) of 5-FU (100 tablets/batch) were prepared by the polymer blends of carbomer and natural oligofructose inulin using direct compression technique. The tablets were obtained by mixing the polymeric excipients along with the drug after sieving (#30 mesh). Then, magnesium stearate (1% w/w) as lubricant, followed by talc (2% w/w) as glidant were further added in the blend. The final powder blend was mixed physically using polythene bag for ten minutes to obtain better flow properties and uniform mixing of the drug. Matrix tablets were compressed manually on single punch tablet press (Cadmach, Ahmedabad, India) using (9.8 mm size) concave punch. Table 1 reflects the composition of core of matrix tablets prepared using polymer in varying ratios with respect to the drug. All the batches (CI10, CI20 and CI30) obtained were evaluated for uniformity of weight, tablet crushing strength using Monsanto's hardness tester (Mac®, Macro Scientific Works Pvt. Ltd., New Delhi, India), friability using Roche's friabilator (Macro Scientific Works Pvt. Ltd., New Delhi, India) and drug content.

Table 1. Composition of core matrix of the uncoated 5-FU tablet batches

Batch	5-FU (%w/w)	Carbopol® 71G-NF (%w/w)	Inulin (%w/w)	Mg stearate (%w/w)	Talc (% w/w)

FUP	33.3	64.6	-	1	2
FUI10	33.3	54.6	10	1	2
FUI20	33.3	44.6	20	1	2
FUI30	33.3	34.6	30	1	2

Mixed film coated colon targeted tablets of 5-FU

Preparation of coating solution

Eudragit ®S-100: ethyl cellulose coating solution

The coating solution containing a mixture of ethyl cellulose and Eudragit® S-00 (6.0% w/v, in a ratio of 2:1) was prepared in a mixture of ethanol and isopropyl alcohol (1:3). A non-aqueous plasticizer, dibutyl phthalate (in a concentration of 10% w/w with density of 1.042-1.045 g/ml at 20°C) was used to provide the flexibility to the film. The solution was stirred for a sufficient period using a mechanical stirrer (2000 rpm) to obtain a clear solution.

Coating of the core tablets

The mixed film coat was applied onto the tablets containing Carbopol®71G-NF and inulin at different levels (2% w/w, 4% w/w and 6% w/w) with the help of Gans® Coater (Gansons® India) at optimized process parameters (i.e., inlet air temperature of 40-50°C; exhaust temperature of 35-40°C, flow rate of 4-5 ml/min, Pan motor speed of 30~rpm). Samples were subsequently collected at regular time intervals and weighed simultaneously till the desired weight gain was obtained.

RESULTS AND DISCUSSION

FOURIER TRANSFORM INFRARED SPECTRA (FTIR)

The FTIR of 5-FU was performed in a KBr disc (compressed into pellets using a hydraulic press) having a weight ratio of a sample and potassium bromide 1:100 mg) using PerkinElmer FTIR spectrophotometer as shown in below figure.

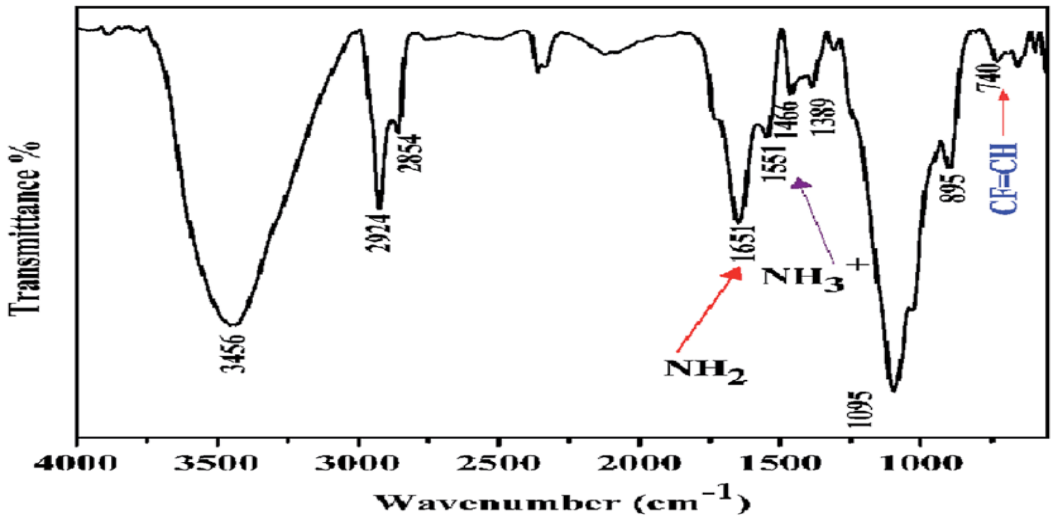

Fig 1: FTIR Spectra of 5-5-FLUOROURACIL

The spectral analysis of pure 5-FU showed characteristic absorption peaks at 3456 cm^{-1} (N-H stretching), 1651 cm^{-1} (C=0 stretching), 1551 cm^{-1} (C=N stretching), 1466 cm^{-1} (C=N stretch), 1389 cm^{-1} (CH in plane) and 740 cm^{-1} (CH out of plane) for the drug as depicted in Fig. 5.44. Moreover, the FTIR studies were also performed to ascertain the compatibility of various excipient-blends with the pure drug. Spectral analysis was carried out using FTIR technique to investigate the formation of new complexes or any chemical changes in the functional moieties of the compounds among blends.

In-vitro intestinal permeability studies

The rate of 5-FU absorption in terms of flux was obtained in small and large segments of pig intestine as shown in Figs. 5.50-5.51 respectively. The permeability of 5-FU was obtained to investigate the absorption pattern in the pig intestine using everted gut sac model and flux (mucosal to serosal) was calculated in both small intestine and large intestine. The concentration of drug per unit area (flux) in the small intestine was observed to be 0.390mg/cm2 as depicted in below Figure 2.

Table 2: Evaluation of flux of 5-FU through small intestinal segments of pig

Time (hr)	Conc./area (mg/cm^2)
1.5	3.063±0.009
2	3.248±0.021

2.5	3.468±0.035
3	3.610±0.042
3.5	3.789±0.018
4	4.012±0.010
4.5	4.235±0.051

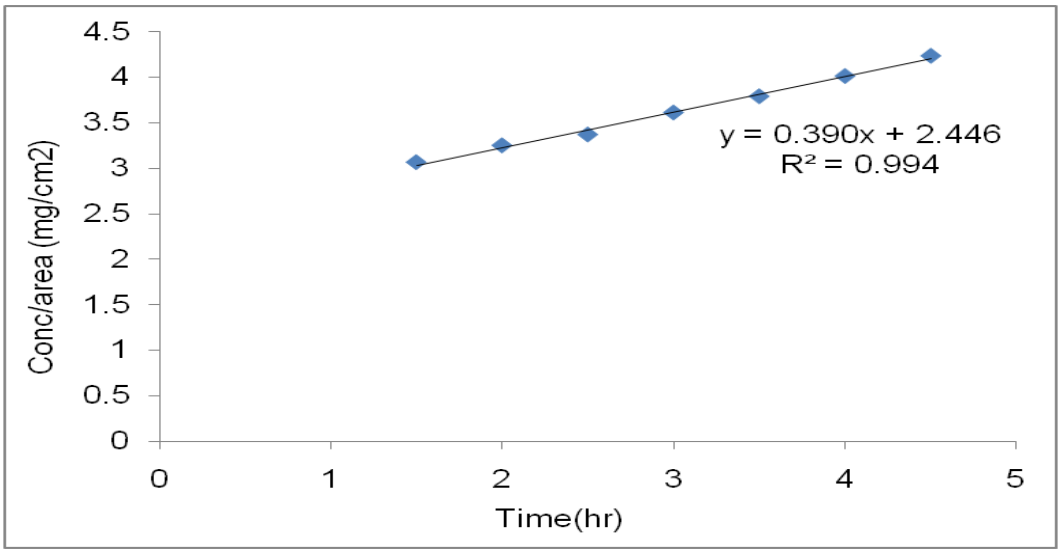

Figure 2: Conc/area versus time graph for evaluation of flux of 5-FU through small intestinal segments of pig

Table 3: Evaluation of flux of 5-FU through large intestinal segments of pig

Time (hr)	Conc./area (mg/cm^2)
4	3.301±0.010
4.5	3.469±0.029
5	3.568±0.056
5.5	3.696±0.038
6	3.803±0.027
6.5	4.027±0.008
7	4.108±0.078

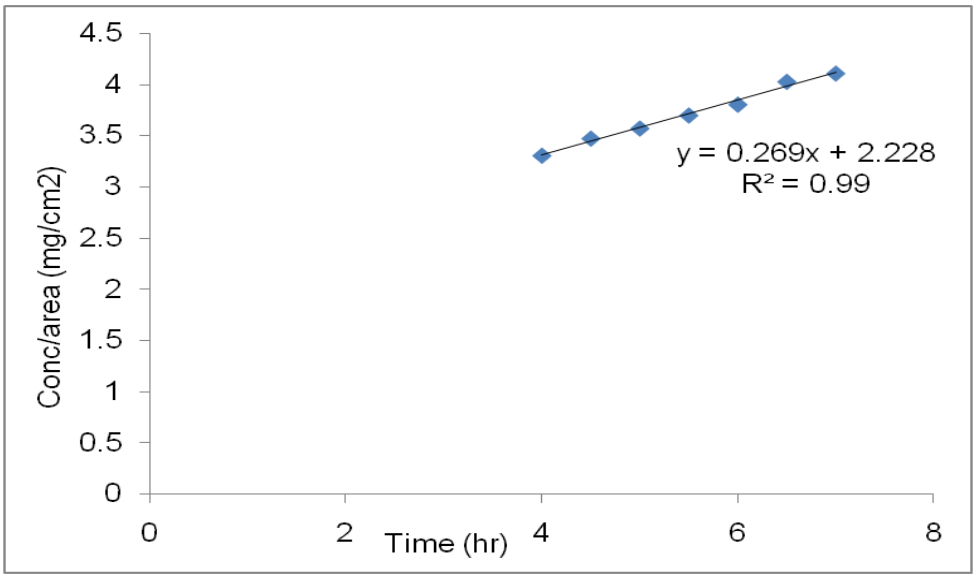

Figure 3: Conc/area versus time graph for evaluation of flux of 5-FU through large intestinal segments of pig

From the permeability studies, it was clearly indicated that higher rate of flux in small intestine may be attributed to the active transport of the 5-FU molecules via Na^+ dependent mechanism. However, in case of large intestine, the mechanism of 5-FU absorption occurred via passive process as reported in literature. Hence, the results from these studies could be exploited to obtain maximum systemic as well as local effect of 5-FU. These studies could be utilized for the effective designing of the formulation approaches to specific target the 5-FU at the colonic region.

Physical evaluation of matrix tablet systems of 5-FU

The core matrix tablets of 5-FU containing MCC were developed for microbially triggered colon targeting system. The parameters for the tablet evaluation such as average weight, hardness, drug content etc. were found to be in within optimum range as shown in Table 91.

Batch FUP (plain tablets of 5-FU) showed high hardness of 10.69±0.62 Kg/cm2 and diameter of 9.9mm. The batch FUP exhibited satisfactory tablet properties such as 0.12% of friability and 99.6±0.02% of drug content. This batch was further modified by adding inulin which gets specifically degraded in the colonic environment. Core matrix tablets of 5-FU (batch FUI10) were fabricated with mixture of 30 mg of inulin and 163.8mg of carbomer. The batch FUI10 showed sufficient hardness at 9.98±0.78Kg/cm2. The friability and drug content of the batch was found to be 0.08% and 98.0±0.02% respectively. Further, batches FUI20 and FUI30 containing 20% w/w of inulin and 30%w/w of inulin exhibited hardness of 9.86±0.39% and 10.33±0.38% respectively. Batches FUI20 and FUI30 exhibited friability of 0.07% and 0.08% respectively. The assay values of these batches FUI20 and FUI30 were observed to be 98.6±0.03% and 100.8±0.02% respectively (I.P., 2007). Physical evaluation of these tablets is depicted in Table 4.

Table 4: Physical evaluation of colon targeted matrix tablets of 5-FU

Batch	Hardness (Kg/cm2) (mean ± S.D) *	Average weight (mg) (mean ± S.D.) *	Diameter (mm)	Friability (%)	Drug Assay (%)
FUP	10.69±0.62	300.9±1.90	9.9	0.12	99.6±0.02
FUI10	9.98±0.78	299.6±1.68	9.9	0.08	98.0±0.02
FUI20	9.86±0.39	300.7±1.71	9.9	0.07	98.6±0.03
FUI30	10.33±0.38	300.5±1.93	9.9	0.08	100.8±0.02

Swelling studies

Carbopol's (such as 71G-NF, granular grade) are the highly popular carbomers used as pH dependent swelling polymers for controlling the drug release in the GI tract. Carbopol's remains in unionized state at acidic pH and becomes ionized at alkaline pH. The batch FUI30 showed 105.19% of high pH dependent swelling at 8 h. Batches FUI30, FUI20 and FUI10 exhibited swelling index of 145.90%, 115.39% and 105.19% at 12 h respectively as shown in Table 5.40. It was observed that concentration of inulin in the matrix influenced the swelling pattern of the tablets. The oligofructose served the purpose of fluid transport into the tablet allowing Carbopol®71G-NF to get exposed to the medium which resulted in swelling.

Table 5: Swelling studies on Carbopol (®71G NF)-inulin based uncoated matrix tablets

Time (hr)	Swelling index (%)			
	FUP	FUI10	FUI20	FUI30
0	0	0	0	0
0.5	13.35	13.35	3.15	13.35
1	23.55	16.75	16.75	23.55
2	33.76	23.55	23.55	33.76
3	43.96	33.76	33.76	43.96
4	54.17	54.17	47.36	54.17
5	59.27	59.27	55.87	69.47
6	64.37	64.37	64.37	84.78
8	74.57	74.57	74.57	105.19
10	84.78	94.98	91.58	125.59
12	94.98	105.19	115.39	145.90
24	145.90	145.90	145.90	145.90

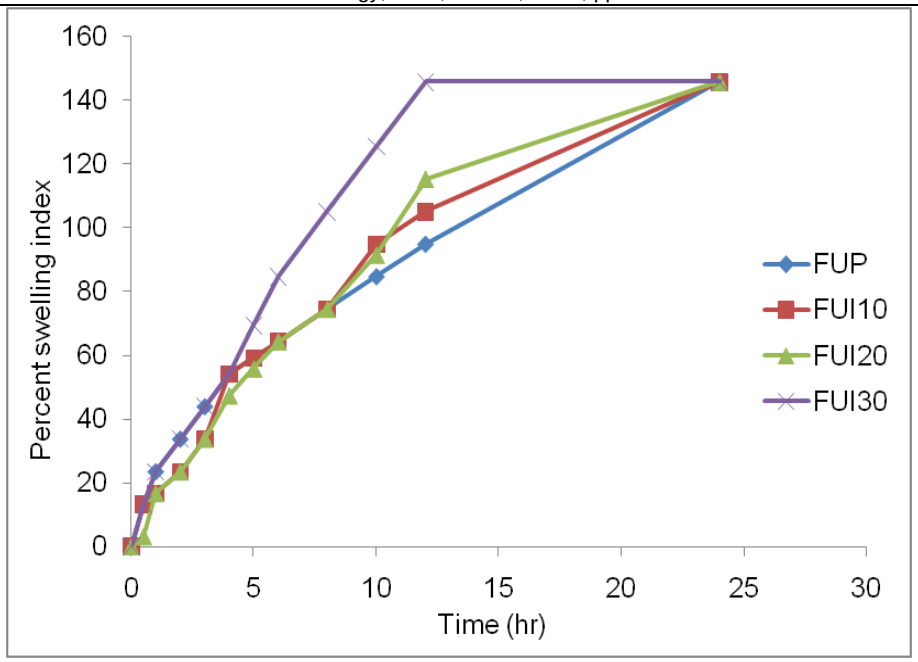

Figure 4: Percent swelling index versus time for tablet formulations, FUP, FUI10, FUI20, and FUI30 (mean ± S.D.; n=3)

From the swelling studies data, it was observed that matrix tablet batches with the increase in the concentration of the inulin or decrease in the concentration of Carbopol®71G in the matrix core, resulted in the augmentation of the swelling index. The entire batches showed swelling index pattern in this order: FUI10<FUI20<FUI30 as shown in Fig. 5.58. This peculiar phenomenon may be justified by the fact that carbomer such as Carbopol®71G is an efficient hydrophilic matrix forming excipient and they enable the uniform dispersion of drugs in polymeric matrix using direct compression. When these carbomer based tablets are placed in contact with dissolution media, the external surface of the tablet becomes hydrated, swells, and forms a gel layer that further controls the release of the drug from the tablets (Noveon® Bulletin 30-31, 2011). With increased Carbopol®71G concentrations in the matrix core, a decrease of the channels dimension inside the matrix tablets consequently lead to decrease of the absorbed water quantity. Further, it was concluded that swelling was the main mechanism influencing the drug release from the matrices composed of Carbopol®71G-NF-inulin mixtures. Furthermore, release exponents obtained for all the batches followed non-fickian diffusion (Table 5).

Dissolution studies on the carbomer-inulin matrix core tablets of 5-FU

An in-vitro study was performed on batch FUP (containing MCC as filler) which showed 24.66±0.67% of drug release after 5 h of the dissolution profile and 81.84±3.93% of total drug release after 24 h as shown in Table 5.41. Batch FUP exhibited slow drug release during the initial 5 h which may be attributed to the water swelling properties of MCC matrix which further increased the diffusion path length and reduced the drug release rate by time.

Table 6: Cumulative percent release from uncoated carbomer-inulin based 5-FU matrix tablets

Time (hr)	Cumulative percent release ± SD (n=3)			
	FUP	FUI10	FUI20	FUI30
0	0	0	0	0
0.5	4.80±1.22	5.31±0.59	6.39±0.24	5.82±0.52
1	8.88±2.72	8.06±0.88	11.56±1.01	9.52±0.32
2	13.58±0.45	15.90±0.76	17.29±0.54	17.40±2.32
3	17.55±0.49	18.74±2.62	21.78±0.53	24.22±2.17
4	20.03±0.44	22.97±0.83	24.09±0.66	28.04±2.44
5	24.66±0.67	26.41±2.47	28.01±0.73	32.68±2.70
6	28.29±0.89	29.85±3.01	32.92±0.81	36.33±3.16
8	38.58±2.17	38.66±3.34	41.01±0.88	47.36±0.51
10	44.10±2.40	46.92±4.67	47.94±2.62	52.84±2.97
12	49.14±3.51	52.40±5.74	55.30±2.59	60.65±3.02
24	81.84±3.93	85.33±6.08	89.92±5.26	100.96±0.95

Furthermore, the batch FUP also exhibited good compression properties and better plasticity. Batches FUI10, FUI20 and FUI30 containing 54.6, 44.6 and 34.6% w/w (of tablet weight) of Carbopol®71G and 10, 20 and 30% w/w (of tablet weight) of inulin respectively were formulated and subjected for in-vitro dissolution studies.

The dissolution profile of the matrix batches (FUI10, FUI20 and FUI30) showed 26.41±2.47%, 28.01±0.73% and 32.68±2.70% of 5-FU release during first 5h and 85.33±6.08%, 89.92±5.26%, and 100.96±0.95% in 24 h respectively (Fig. 5).

It was observed that all the batches showed higher rate of drug release when the concentration of inulin was gradually increased or the concentration of Carbopol®71G decreased in the subsequent batches (viz. FUI10, FUI20 and FUI30) by replacing MCC. Moreover, all the batches (FUI10, FUI20 and FUI30) showed more than >20% of drug release after 5h and exhibited incomplete drug release after 24 h except batch FUI30 (Fig. 5).

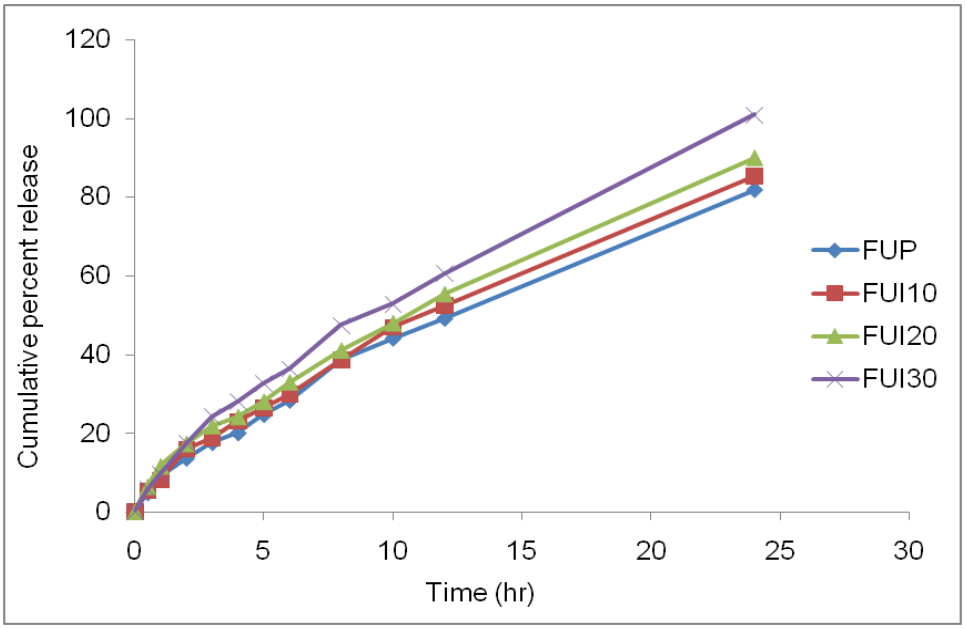

Figure 5: Cumulative percent release versus time for tablet formulations, FUP, FUI10, FUI20, and FUI30 (mean ± S.D.; n=3)

The mechanism of drug release and its kinetics was also evaluated by subjecting the dissolution profiles of all the matrix tablet batches (FUP, FUI10, FUI20, and FUI30) to various release models as shown in Table 6. The values of drug release exponents and coefficient of correlation were obtained through mathematical modelling (Zero order, First order, Higuchi model, Hixon Crowell cube root law, and Korsmeyer- Peppas model). Based on these fitting models, the magnitude of the release component 'n' indicates the release mechanism (i.e. Fickian diffusion, anomalous transport, or super case II transport). The value of n=0.45 reveals Fickian (case I) release; >0.45 but <0.89 indicates non-Fickian (anomalous) release; and >0.89 indicates super case II type of drug release. All the 5-FU matrix batches showed best fit into Korsmeyer-Peppas model with coefficient of regression values between 0.996 to 0.998 along with 'n' values in the range of 0.814-0.899 (Table 6). From this data, it was concluded that the matrix formulations of 5-FU exhibited non-Fickian (anomalous) type of release.

Table 7: Comparative profile of various drug release models, best fit models for the 5-FU tablet batches

Batch	Release models										
	Zero order		First order		Higuchi		Korsmeyer-Peppas		Hixon-Crowell		Best fit model
	k_0	R_0	k_1	R_1	k_h	R_h	n	R_k	k_s	R_s	
FUP	3.208	0.989	-0.09	0.854	13.93	0.901	0.886	0.997	-0.098	0.897	Peppas
FUI10	3.304	0.989	-0.09	0.861	14.50	0.917	0.869	0.997	-0.097	0.889	Peppas
FUI20	3.496	0.991	-0.09	0.865	15.53	0.932	0.814	0.996	-0.094	0.918	Peppas
FUI30	4.256	0.996	-0.09	0.888	17.70	0.899	0.899	0.998	-0.117	0.889	Peppas

Mixed film coated tablets of 5-FU with Eudragit®S-100: ethyl cellulose (1:2)

Due to their high solubility and swelling properties of polymers in aqueous media, film coatings prepared with cellulosic polymer like ethyl cellulose alone are unable to prevent the release of drugs from coated dosage forms during their transit through the stomach and the small intestine. Thus, various authors have suggested to use coatings prepared from cellulosic (Aquacoat® ECD30, Surelease®) or acrylic (Eudragit®S-100, L-100 or RS30D) insoluble polymer aqueous dispersions incorporating appropriate amounts of pectin's or calcium pectinates or ethyl cellulose, to prepare more suitable forms for targeting drugs to the colon. Hence, to minimize the drug release in the upper segments of the GI tract, mixed film coating (Eudragit®S-100 and ethyl cellulose) was carried out on the above-mentioned batches i.e. FUI10, FUI20 and FUI30 at different coating levels for a site-specific designing of the microbially triggered colon targeted system.

Matrix tablets containing the drug, 5-FU, and blend of polymers (inulin and Carbopol®71G-NF in ratios of 5.46:1, 2.23:1, 1.15:1 respectively) coated with a mixed film of ethyl cellulose and Eudragit®S-100 (2:1) were prepared. These systems would provide a lag phase of 5 h to prevent the premature drug release in upper segment of GI tract ensuring the colonic delivery of the drug. Further, Carbopol®71G-NF will act as release retardant and inulin will provide the degradation to the matrix core by colonic microflora ensuring the release of high proportion of 5-FU release in the colonic region. Thus, coating with the blend of polymers (ethyl cellulose and Eudragit®S-100) will provide the benefits of both polymers like enteric coating and release retardant respectively.

Table 8: Cumulative percent release from mixed film coated carbomer-inulin based 5-FU matrix tablet batches (Coat weight level 2%)

Time (hr)	Cumulative percent released ± SD (n=3)		
	FUI10C2%	FUI20C2%	FUI30C2%
0	0	0	0
0.5	4.80±0.789	0	0
1	7.87±0.656	0.314±0.22	0
2	15.90±2.25	0.719±0.016	0.102±0.072
3	22.83±2.13	2.70±1.02	2.75±1.49
4	25.76±0.867	3.06±0.364	7.01±3.67
5	26.73±0.808	6.64±0.872	11.75±4.20
6	27.60±0.760	9.21±1.37	15.49±4.72
8	36.92±2.19	17.57±3.16	23.64±5.23
10	42.92±3.25	25.96±2.55	26.16±4.17
12	50.08±5.01	37.76±2.95	34.20±5.24
24	82.59±2.83	100.73±1.94	100.57±4.31

Batches FUI10, FUI20 and FUI30 were coated at coat weight level of 2% w/w, 4% w/w and 6% w/w respectively. The in-vitro dissolution studies of the mixed film coated batches
(FUI10C, FUI20C and FUI30C) at various coating thickness levels has been illustrated in Table 5.43. Initially these batches were coated to obtain a 2% w/w weight gain and designated as FUI10C2%, FUI20C2% and FUI30C2% respectively to retard the drug release in initial hours. It was observed that batch FUI10C2% could not retard the drug release in initial 5 h and exhibited 26.73±0.808% of 5-FU release (Fig. 6). However, batches FUI20C2% and FUI30C2% showed strong retardation during initial 5 h releasing 6.64±0.872% and 11.75±4.20% of 5-FU respectively (Fig. 5.60). Moreover, complete drug release occurred at 24 h in all the batches.

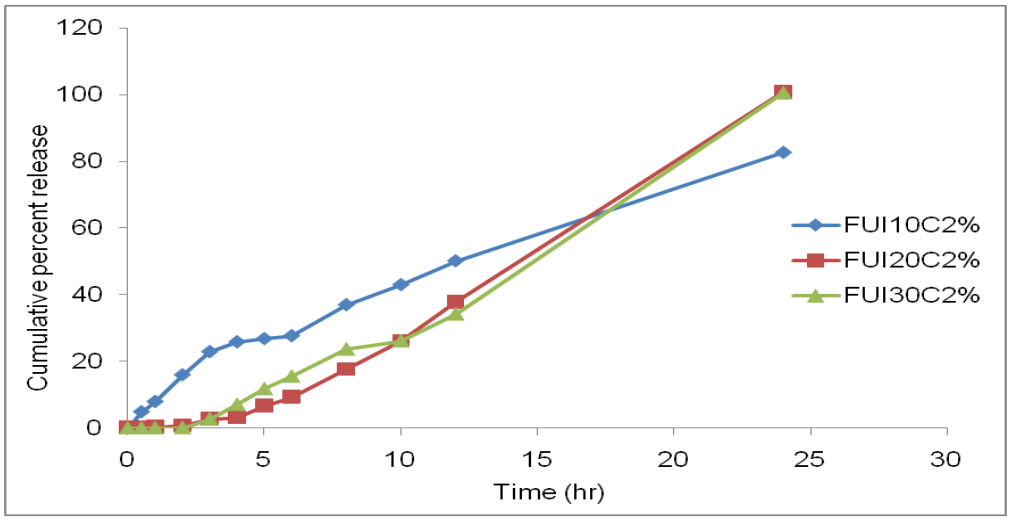

Figure 6: Cumulative percent release versus time for tablet formulations, FUI10C2%, FUI20C2%, and FUI30C2% (mean ± S.D.; n=3)

On increasing the coating level to 4% w/w, batches FUI10C4% and FUI20C4% did not showed complete drug release of drug at 24 h, but batch FUI30C4% which displayed complete release of drug at 24 h and a significant decrease in drug release was observed in initial 5 h (<1%).

Table 9: Cumulative percent release from mixed film coated carbomer-inulin based 5-FU matrix tablet batches (Coat weight level 4%)

Time (hr)	Cumulative percent released ± SD (n=3)

	FUI10C4%	FUI20C4%	FUI30C4%
0	0	0	0
0.5	0	0	0
1	0.292±0.459	0.047±0.008	0
2	0.705±0.087	0.052±0.04	0
3	1.58±0.238	0.002±0.01	0
4	3.02±0.397	0.002±0.01	0
5	5.93±0.815	0.052±0.08	0.955±0.63
6	7.99±1.43	0.092±0.25	2.99±2.16
8	13.79±2.47	0.908±0.82	7.48±3.55
10	24.71±3.71	3.98±0.986	14.71±2.18
12	31.84±6.61	7.66±0.503	21.94±5.07
24	72.56±6.39	51.99±4.69	100.84±4.60

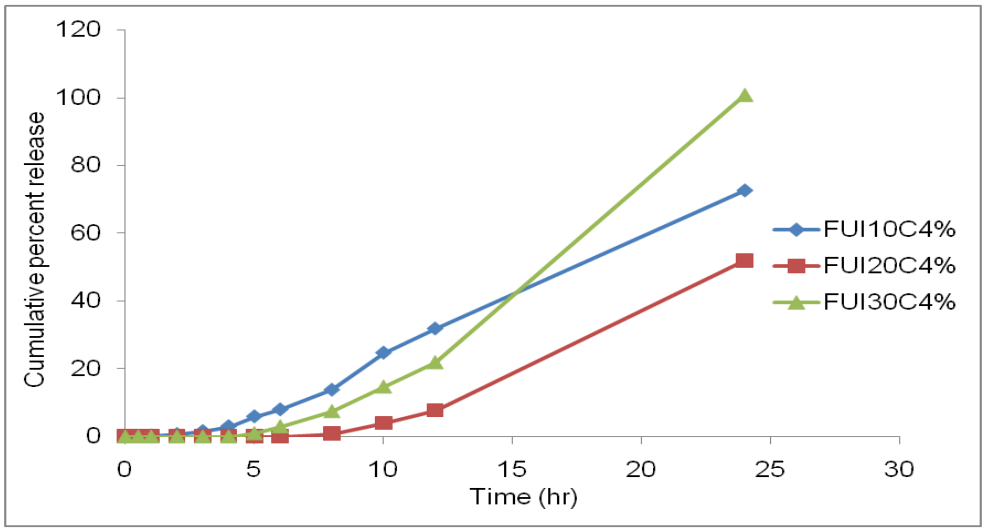

Figure 7: Cumulative percent release versus time for tablet formulations, FUI10C4%, FUI20C4%, and FUI30C4% (mean ± S.D.; n=3)

Further, on increasing 6% w/w coat weight, all the batches (FUI10C6%, FUI20C6% and FUI30C6%) exhibited high retardation and up to 10-20% release in 10-12 h and a very low percent of the drug was released at 24 h (Fig. 7). Hence, these batches were not selected for further studies and thus rejected.

Table 10: Cumulative percent release from mixed film coated carbomer-inulin based 5-FU matrix tablet batches (Coat weight level 6%)

Time (hr)	Cumulative percent released ± SD (n=3)		
	FUI10C6%	FUI20C6%	FUI30C6%
0	0	0	0
0.5	0	0	0
1	0	0	0
2	0.786±0.06	0.010±0.02	0.074±0.02
3	0.620±0.25	0	0.005±0.00
4	2.34±0.403	0.111±0.054	0.0078±0.02
5	3.74±0.628	0.096±0.063	0.083±0.050
6	5.12±0.488	0.070±0.071	0.249±0.099
8	8.64±0.226	0.002±0.00	0.388±0.285
10	13.42±1.15	0.002±0.00	2.05±2.30
12	18.63±4.72	0.002±0.00	6.55±2.82
24	47.55±5.50	15.65±5.33	59.17±4.47

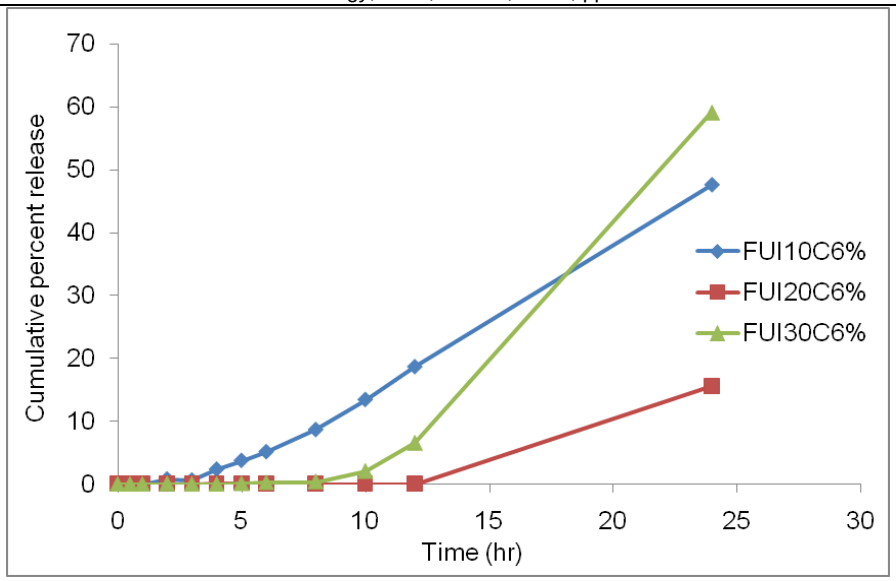

Figure 8: Cumulative percent release versus time for tablet formulations, FUI10C6%, FUI20C6%, and FUI30C6% (mean ± S.D.; n=3)

In-vitro drug release in rat cecal contents

Finally, among all the batches FUI30C2% and FUI30C4% were selected for dissolution test in presence of rat caecal content to achieve the complete drug release. As the both batches FUI30C2% and FUI30C4% contained fraction of inulin (specifically fermented and degraded by colonic microbial inulinases), complete release of the 5-FU was expected in the buffer media containing rat caecal contents. Further, the in-vitro dissolution studies were performed with both mixed films coated batches in the presence of rat caecal contents, which was further compared with the data obtained from in-vitro dissolution studies carried out in the normal buffer conditions (i.e. without rat caecal contents). The dissolution profile of mixed film coated batches (FUI30C2% and FUI30C4%) was subjected to rat caecal content studies during the interval of 5-24 h. However, the initial phase of dissolution was performed in 0.1 N HCl for 3 hr in pH 6.8 buffer till 5 hr interval. The samples were analysed by validated RP-HPLC method. Furthermore, the interference of 5-FU with the rat caecal content media was evaluated using a validated RP-HPLC.

During the in-vitro release studies of batch FUI30C2% the samples analysed by RP-HPLC showed 5.16±0.56% of drug release in initial 5 h and 100.22±2.50% of drug release in 24 h in the presence of rat caecal contents (Table 8 and Fig. 9). Further, it was quite clear from the dissolution profile of the batch FUI30C2% exhibited a lag in drug release during the 10 and 12 h and only released 40.85±0.89% and 54.18±3.67% of drug in the absence of rat caecal contents respectively. However, the enhancement in drug release (46.77±0.82% and 64.31±2.36%) during the 10 h and 12 h intervals respectively was observed in the presence of rat caecal content medium. This investigation confirmed the susceptibility of oligosaccharide inulin to the colonic enzymes (inulinases) secreted by host microflora in the rat intestine. Hence these results were in accordance with the earlier literature reports suggested by various authors (Havenaar et al., 1999; Sinha and Kumria, 2003; Sinha et al., 2004). The statistical analysis (student t-test) of the dissolution studies (with or without rat caecal contents) showed significant difference ($P<0.05$) in the drug release.

Table 11: Cumulative percent release from batch FUI30C2% with and without rat cecal content medium (analyzed by RP-HPLC)

Time (hr)	Cumulative percent release± SD (n=3) from batch FUI30C2%	
	Without rat caecal content	With rat caecal content
0	0	0
1	0.70±0.25	0.25±0.28
2	4.58±0.38	0.98±0.69
3	7.97±0.40	1.20±1.16
4	11.91±0.21	4.20±0.59
5	16.62±0.86	5.16±0.56
6	21.64±2.05	18.68±0.78
8	33.17±3.75	33.23±1.14
10	40.85±0.89	46.77±0.82
12	54.18±3.67	64.31±2.36
24	100.52±6.25	100.22±2.50

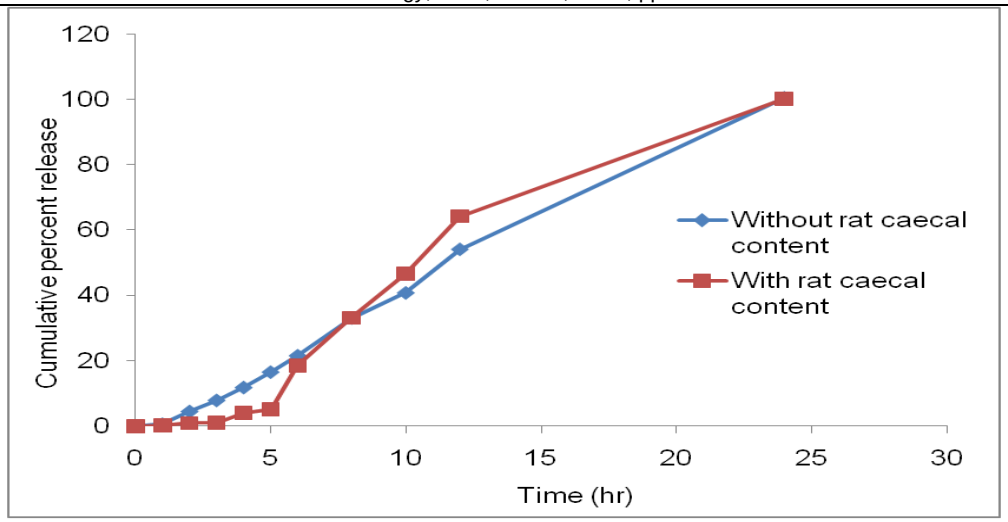

Figure 9: Comparative in-vitro dissolution profile of coated batch FUI30C2% with or without rat cecal contents (mean ± S.D.; n=3)

Similarly, another selected batch FUI30C4% was subjected to in-vitro release studies using rat caecal contents and the samples obtained at different intervals were analysed using RP-HPLC. The batch FUI30C4% showed 7.41±1.48% of drug release after the initial 5 h of dissolution study as shown in Table 11. Further, it was observed that, mixed film coated batch exhibited 35.42±0.37% and 50.13±2.87% drug release at 10 h and 12 h in the presence of rat caecal contents respectively (Table 11). This study showed that 100.22±2.50% and 100.32±3.21% of drug was released from FUI30C2% and FUI30C4% respectively between 5 h and 24 h ensuring that maximum amount of the drug was released in the colonic region. Hence, these batches showed better release profile in presence of rat caecal content which further ensured the biodegradability of inulin by the inulinases secreted by colonic microflora. The statistical analysis (Student's t-test) of the data (release with and without rat caecal contents) showed significant difference ($p < 0.05$) in the drug release (Fig. 5.64). Hence, it was inferred that batches FUI30C2% and FUI30C4% showed optimum release during the initial 5 h and complete drug release at 24 h (Figs. 5.63 and 5.64). Further, to obtain uniform drug release (due to lag in the drug release), rat caecal studies were performed. Furthermore, 1.86-fold and 1.15-fold increase in drug release at 10 h and 1.88-fold and 1.19-fold increase at 12 h in both batches FUI30C2% and FUI30C4% respectively was obtained due to enzymatic degradation of inulin in the matrix by colonic microflora

Table 12: Cumulative percent release from batch FUI30C4% with and without rat cecal content medium (analyzed by RP-HPLC)

Time (hr)	Cumulative percent release± SD (n=3) from batch FUI30C4%	
	Without rat caecal content	With rat caecal content
0	0	0
1	0	0.88±0.20
2	0	0.76±0.65
3	0.42±2.16	3.46±1.60
4	2.69±0.59	5.43±0.58
5	4.64±0.56	7.41±1.48
6	6.58±0.78	10.03±1.09
8	12.78±2.14	21.06±0.78
10	19.53±1.82	35.42±0.37
12	27.20±2.36	50.13±2.87
24	96.84±1.65	100.32±3.21

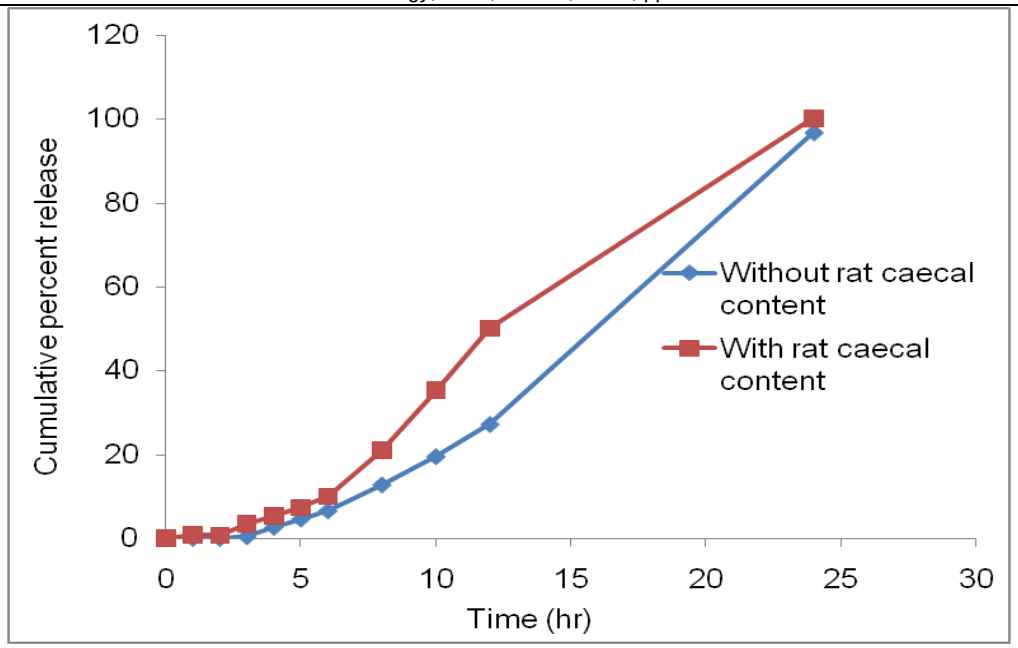

Figure 10: Comparative in-vitro dissolution profile of coated batch FUI30C4% with or without rat cecal contents (mean ± S.D.; n=3)

CONCLUSION

Thus, colon targeted drug delivery systems were prepared using various formulation approaches. The physicochemical characterization of 5-FU was done by pre formulation studies. Various studies such as ultra-violet (UV) absorption spectrum in different media (0.1N HCl, phosphate buffer pH 6.8), IR spectral analysis, DSC studies were carried out. Further, DSC thermograms showed characteristic endothermic peak at 287°C, which is an indicative of the melting point of a crystalline drug. Furthermore, drug-excipient interactions were / also studied in the physical mixtures (in ratio of 1:1) used for the preparation of colonic dosage forms (tablets or pellets). It was observed that all the DSC thermograms retained the endothermic peak of 5-FU indicating, no interaction with the formulation excipients. The rate of 5-FU absorption in terms of flux was also studied in small and large intestinal segments of the pig. The permeability of 5-FU (concentration of drug per unit area or flux) in small and large intestine was obtained to be 0.390mg/cm2 and 0.269 mg/cm2 respectively. Standard plots of 5-FU in 0.1 N HCl and phosphate buffer pH 6.8 showed good linearity over the concentration range from 0-16 µg/ml. The λmax of 5-FU for both 0.1 N HCl and phosphate buffer pH 6.8 was observed to be 274 nm respectively. The values of E (1%, 1 cm) for 5-FU in 0.1 N HCl and phosphate buffer pH 6.8 were found to be 580 and 570, respectively. Moreover, standard plots of 5-FU in water, 0.1 N HCl, phosphate buffer (pH 6.8) and rat caecal medium were obtained using a RP-HPLC method already validated in our laboratory. The standard plots showed good linearity over a concentration range of 10-100 µg/ml. A very high correlation (R^2-0.9999, 0.9999, 0.9998, 0.9997) for 5-FU in water, 0.1 N HCl, phosphate buffer pH 6.8, and rat caecal medium with a slope of 70919, 50208, 60154, and 27167 respectively.

To achieve site specific drug delivery to the colon, one of the approaches used was microbial triggered drug delivery system. These systems have become an important tool for the effective drug delivery of the important molecules such as 5-FU. As 5-FU chemotherapy is highly hampered by its dose associated toxicity thorough i.v, i.p and s.c. administration. Thus, targeting of 5-FU at the specific site using a microbially trigged system could be viable option. For the development of 5-FU colonic ystems, core matrix tablet batch of 5-FU containing MCC (batch FUP) was prepared. Other matrix tablet batches containing inulin were prepared to impart degradability in the matrix system so that it gets specifically degraded by the enzymes secreted by colonic microflora. Matrix tablet batches FUI10, FUI20, and FUI30 containing mixture of 10, 20 and 30% of inulin (of total tablet weight) and 54.6, 44.6 and 34.6% w/w of Carbopol 71G were prepared and evaluated for various compendia and non-compendial tests. All the batches showed satisfactory tablet properties. Further, these batches were also evaluated for swelling index studies. Batches FUI10, FUI20, and FUI30 showed 26.41±2.47%, 28.01±0.73% and 32.68±2.70% of drug release in 5 h of the dissolution. Batches FUI10, FUI20, and FUI30 did not show sufficient retardation in 5 h and showed incomplete drug release except batch FUI30. The mechanism of the drug release and kinetics was evaluated using mathematical drug models. It was observed all the batches FUI10, FUI20, and FUI30 followed Peppas model and the values of ‘n’ as 0.869, 0.814, and 0.899 respectively. However, these batches were not able to deliver drug specifically to the colon, hence further optimization was carried out by coating of these batches using a mixed film polymer.

Mixed film coating approach was utilized for the colonic drug delivery of core matrix tablets of 5-FU batches FUI10, FUI20, and FUI30. Mixed film coated batches FUI10C, FUI20C, and FUI30C were coated with ethyl cellulose: Eudragit®S-100 (in a ratio of 2:1) at 2, 4, and 6% w/w of coating levels. Batches FUI10C2%,

FUI20C2% and FUI30C2% showed 26.73±0.808%, 6.64±0.872% and 11.75±4.20% of drug release in initial 5 h and more than 80% of drug was released after 24h of the dissolution. Batches FUI10C4%, FUI20C4% and FUI30C4% showed 5.93±0.815%, 0.052±0.08%, and 0.955±0.63% of drug release in 5h and released 72.56±6.39%, 51.99±4.69% and 100.84±4.60% of drug after 24 h. Further, batches FUI10C6%, FUI20C6% and FUI30C6% exhibited very poor release due to high retardation (by increased coat weight) even after 24 h of the dissolution.

Batch FUI30C2% and FUI30C4% which showed 11.75±4.20% and 0.955±0.63% of drug release in 5h and complete release in 24 h were found to be suitable for the colonic drug delivery. Furthermore, these coated batches also showed slow drug release of 26.16±4.17% and 14.71±2.18% in 10hr whereas 34.20±5.24% and 21.94±5.07% of drug release in 12hr. The lag in drug release at these time intervals were optimized by subjecting these batches to in-vitro release studies in the presence of rat caecal contents.

Because of presence of inulin in the matrix core, these tablets would be targeted at specific site and degraded by the enzymes secreted by colonic microflora. The in-vitro dissolution samples were analysed using a RP-HPLC method. Batches FUI30C2% showed 40.85±0.89% of drug release without rat caecal contents and 46.77±0.82% of drug release in the presence of rat caecal contents at 10 hr interval. Further, 54.18±3.67% of drug was released without rat caecal contents whereas 64.31±2.36% of release was obtained in the presence of rat caecal contents at 12 hr interval.

Similarly, batch FUI30C4% showed 19.53±1.82% of drug release without rat caecal content whereas 35.42±0.37% of drug release was obtained in the presence of rat caecal contents at 10 h of the dissolution. Furthermore, 27.20±2.36% of drug release was found without rat caecal contents whereas 50.13±2.87% of drug release was obtained at 12 h interval of the dissolution study. The statistical analysis of the data (Student's t-test) (release with and without rat caecal contents) showed significant difference in the drug release ($p<0.05$). Mixed film coated tablet batches (based on microbially triggered approach) of 5-FU (FUI20C2% and FUI30C4%) coated at 2% and 4% w/w of coating level using ethyl cellulose: Eudragit®S-100 (in a ratio of 2:1) were successfully developed for the colonic drug delivery.

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