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Research Article

DEVELOPMENT AND IN VITRO EVALUATION OF EUDRAGIT RS100 AND INULIN COATED PECTIN MATRIX TABLETS OF 5-FLUOROURACIL FOR COLON TARGETING

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ABSTRACT

Various formulations of pectin matrix tablets containing 5-Fluorouracil (5-FU) coated with combination of Eudragit RS100 and inulin were prepared and evaluated for release of drug in the colon, which is a prerequisite for the effective treatment of colorectal carcinoma. *In vitro* dissolution studies of formulations F1, F2, F3 and F4 containing 30%, 45%, 60% and 75% by weight of pectin respectively revealed that formulations F1, F2, F3 and F4 released the entire drug after 3, 4, 6 and 11 hours of the study. The cumulative percentage release data of formulations F5, F6, F7, F8 and F9 containing 75% by weight of pectin in the matrix coated with combination of Eudragit RS 100 and inulin in the ratio of 100%, 90%: 10%, 80%: 20%, 70%: 30% and 60%: 40% revealed that F9 is the best formulation as it released only 13.2±3.21% of drug after 5 hours. To further retard the initial release, formulations F10 and F11 were coated to obtain a weight gain of 10.85% and 12.91% of total weight of tablets respectively. Formulation F11 showed the best release data as it released 8.5±2.58% of drug after 5 hrs, which was less than that released by other formulations. The complete drug release from formulation F11 was further tested *in vitro* in the presence of rat caecal contents and it was observed that 87.1±3.5% of drug was released after 24 hrs in the presence of rat caecal content.

Key words: 5-Fluorouracil, Pectin, Inulin, Eudragit RS100, Colon drug delivery.

INTRODUCTION

Targeting of drugs specifically to colon is advantageous for the treatment of several colonic diseases like Inflammatory bowel diseases (Chron's disease and ulcerative colitis), irritable bowel syndrome and colon cancer. This site-specific delivery to colon reduces the side effects associated with the therapy. Colon, as a site, offers distinct advantages on account of near neutral pH, a much longer transit time, reduced digestive enzymatic activity and a much greater responsiveness to absorption enhancers. These criteria favor this distal part of the GIT as a site for delivery of various drug molecules including proteins and peptides[1].

As colon is the distal segment of the large intestine, hence targeting the drug to the colon is very problematic. Though the rectal route can also be accessed for drug delivery to the colon, it further offers limitations of limited transit of the drug in the intestinal passage. Furthermore the rectal route of drug delivery is not convenient and acceptable for patients. Thus, targeting of the drug to the colon after oral administration is the most convenient and advantageous route for the delivery of a wide array of the drugs. An oral colon targeted drug delivery system (CDDS) prevents the premature release of the drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the colon following oral administration[2].

Various systems have been developed for colon specific drug delivery, which involves the presence of a triggering element in the system that exploits the physiological changes in the colon[3]. Many delivery systems have been designed which rely on pH, transit time, pressure and microflora to initiate drug release in the colon[4]. The pH and time dependent drug delivery systems still lack the site specificity because of large variations in pH and gastric emptying time. But microbially triggered drug delivery systems possess the excellent site specificity and a large number of polysaccharides recently have been proposed for development of colon specific drug delivery systems[5].

Pectin is an anionic polysaccharide, which remains intact in the physiological environment of the stomach and the small intestine, but is degraded by the bacterial inhabitants of the human colon[6, 7]. Various matrix systems using different types of pectins have been developed for oral formulations intended to release their active substance in the colon[8-10].

Inulin is a natural polysaccharide that is not hydrolyzed by gastric and small intestinal enzymes but is completely hydrolyzed by colonic bacteria *bifidobacteria* [11, 12] which constitute up to 25% of the normal gut flora in man [13].

Eudragit RS100 is cationic copolymers of ethyl acrylate, methyl methacrylate and a low content of a methacrylic acid ester with quaternary ammonium groups (trimethylammonioethyl methacrylate chloride). This is a water impermeable, pH-independent polymer, which has been widely used in the development of various sustained release formulations.

For several decades, 5-Fluorouracil has been used as a drug of choice for the treatment of colon cancer[14]. Site-specific delivery of 5-FU to the colon overcomes the side effects associated with the parenteral delivery of the drug, which include gastrointestinal toxicity, hematological and neural disorders and cardiac manifestations. Due to the enormous advantages offered by site-specific delivery of drugs to the colon an attempt was made to design a colon-specific drug delivery system for 5-FU for the treatment of colorectal carcinoma.

The objective of the present investigation was to design a single unit oral colon-specific formulation for 5-FU using pectin in the matrix coated with a combination of natural polysaccharide inulin and sustained release polymer Eudragit RS 100.

MATERIALS AND METHODS

5 Fluorouracil was purchased from Himedia Laboratories Limited, Mumbai and was used as received. Pectin and inulin were aiso purchased from Himedia Laboratories Limited, Mumbai. Eudragit RS100 was obtained as a gift sample from Panacea Biotec, Lalru. Other ingredients used were of analytical grade.

Differential Scanning Calorimetry (DSC) studies

DSC studies were performed to investigate the physicochemical compatibility between 5-Fluorouracil and various excipients (Pectin,

Inulin, and Eudragit RS100) used in tablet manufacturing. Themograms of 5-FU, 5-FU: pectin and 5-FU: inulin: Eudragit RS100 was obtained over the temperature range of 20-350 °c. The thermograms of 1:1 physical mixtures of these excipients with 5-FU were obtained and peaks of mixtures were compared with peaks of pure drug

Preparation of colon targeted drug delivery system

Matrix tablets of 5-fluorouracil

Different batches of matrix tablets of 5-Fluorouracil F1, F2, F3, and F4 containing 30%, 45%, 60% and 75% by weight of pectin were prepared by direct compression method. Microcrystalline cellulose was used as the directly compressible vehicle, magnesium stearate (1%) as the lubricant and talc (2%) as the glidant. Drug (125 mg), different concentrations of pectin and spray-dried lactose was mixed in dry state followed by sieving to obtain a uniform blend. Finally, the lubricant and glidant were added and the blend was further mixed for fifteen minutes and then compressed into tablets on single punch machine.

Coated matrices of 5-Fluorouracil

Preparation of coating solution

The coating solution of Eudragit RS 100 was prepared by dissolving the granules of Eudragit RS100 in 9:1 ratio of isopropyl alcohol and distilled water. Dibutyl phthalate (10%w/w) was used as the plasticizer. The solution was stirred for sufficient period of time in order to obtain a clear solution. Inulin solution was prepared by dissolving the specific amounts of inulin powder in warm distilled water. Inulin solution was added to Eudragit RS100 solution with continuous stirring in order to obtain solutions with different compositions of Eudragit RS100 and inulin in 100%:0%, 90%: 10%, 80%: 20%, 70%: 30% and 60%: 40%[15].

Coating of the core tablets

The formulations F5, F6, F7, F8 and F9 were obtained by coating the matrix tablets of 5-FU containing 75% w/w of pectin with combinations of Eudragit RS100 and Inulin in ratios of 100:0, 90:10, 80:20, 70:30 and 60:40 respectively in order to obtain 9% w/w of coating**.** The coating of matrix tablets was done in a conventional coating pan with an inlet temperature of 35-45º C. The coating process was continued in order to obtain a required level of coat weight[16].

Formulations F10 and F11 were obtained by coating matrix tablets with combination of Eudragit RS100 and inulin in the ratio of 60%: 40% to obtain 10.85 and 12.91% w/w of coating.

Evaluation of the tablets

Physical evaluation of tablets

Tablets were evaluated for various parameters such as diameter, thickness, hardness, friability and drug content. Diameter and thickness of all batches were noted with the aid of calibrated vernier calipers, in order to determine the uniformity in the batches. Hardness of the tablets was determined using Monsanto hardness tester. Friability was determined using Roche friability tester for all the batches. The drug content was assayed by taking one tablet from each batch. The tablet was weighed and powdered in a mortar and pestle. Powder equivalent to 50 mg of the drug was weighed and dissolved in 50 ml of 0.1N HCl, in order to obtain a solution of 1 mg/ml. The solution was sonicated for 5 minutes. It was centrifuged for 10 min at 4000 rpm. After centrifugation the supernatant was taken and was subsequently diluted with 0.1N HCl. The drug content was calculated by analyzing the sample at 266 nm with UV spectrophotometer[17] (UV-160A, SHIMADZU).

***In vitro* release studies**

Dissolution test was carried out on all the formulations according to USP XXVII method for delayed release tablets (Method A). Dissolution studies were carried out using USP apparatus type-I i.e. basket type (LABINDIA DISSO 2000, Digital tablet dissolution test apparatus) at 100 rpm and at a temperature of 37±0.5 ºC. Initial studies were carried out in 750 ml of 0.1N HCl (pH: 1.2) for 2 hours followed by addition of 250 ml of 0.2 M trisodium orthophosphate, in order to obtain a final pH of 6.8 in the medium for 3 hours and then pH was adjusted to 6.0 and studies were carried out for further 6 hours. The pH was adjusted with the aid of 2N HCl or 2N NaOH. Samples were withdrawn at predetermined time intervals and replaced with fresh media. The samples were then analyzed using UV-spectrophotometer at λ_{max} of 266 nm[17]. Further, the *in vitro* studies of the final formulation F11 were carried out in the presence of rat caecal content.

Swelling Index Studies

Swelling index studies were carried on the batches F5, F6, F7, F8, F9, F10, and F11. The degree of swelling was observed by placing the tablets in a 150 ml beaker containing 90 ml of 0.1N HCl for initial two hours followed by addition of 30 ml of 0.2 M trisodium orthophosphate in order to obtain a final pH of 6.8 in the medium for further two hours and then pH was adjusted to 6.0 and the swelling studies were carried out for further six hours. The temperature was maintained at 37±0.2 ºC throughout the studies. Changes in the diameter of the tablets were recorded at regular time interval[18, 19].

The degree of swelling in the tablets was calculated using the following equation:

$$\text{Swelling Index} = [D_T - D_0] / D_0 \times 100$$

Where, D_T = diameter (mm) of the tablet at time T (h)

D_0 = diameter (mm) of the tablet at 0-time.

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC):

By using this method, an evaluation of the compatibility between drug and excipients was measured by a simple operation in a short time. Thermograms of 5-FU, pectin, inulin, Eudragit RS100 and their combinations over the temperature range of 20-350 °c are shown in figures 6-8. An endothermic peak was observed at 283.45°c in case of pure drug 5-FU. Thermograms in case of pure pectin are obtained at 172.02 and 244.63 °c, in case of inulin at 168.33 and 228.56 °c and in case of Eudragit RS100 at 59.09 and 195.48 °c. The thermograms of combinations 5-FU: pectin and 5-FU: inulin: Eudragit RS 100 is obtained at 282.59 and 278.55 °c. This data suggest that there is no incompatibility between drug and various excipients as endothermic peaks in case of combinations are obtained at almost same temperature as that of pure drug. Hence, based on the results of DSC, it was concluded that the drug was compatible with the other excipients and would result in a stable formulation.

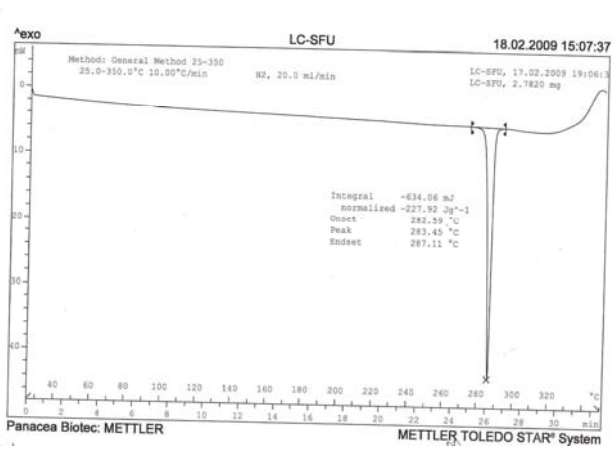

Fig. 6: DSC plot of 5-Fluorouracil

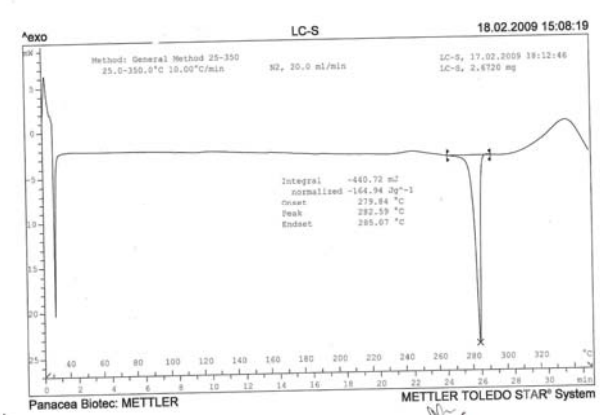

Fig. 7: DSC plot of 5-Fluorouracil:Pectin

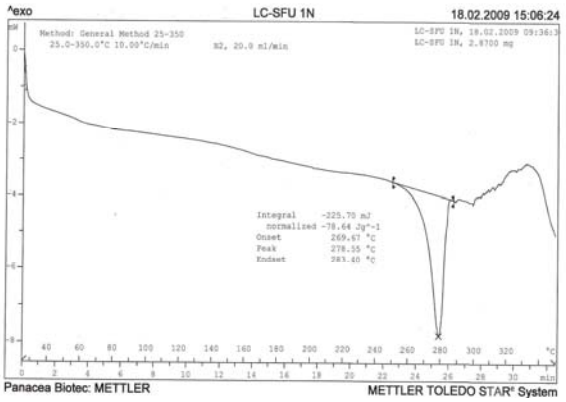

Fig. 8: DSC plot of 5-Fluorouracil:Inulin:Eudragid RS 100

Table 1: Values of physical parameters and assay of 5-Fluorouracil tablets

Formulation Code	Hardness (kg/m^2)	Diameter (mm) ± S.D	Thickness (mm) ± S.D	Friability (%)	Assay (%)
F1	6.1	11.7±0.01	2.99± 0.005	0.8	99.85
F2	6.7	11.8±0.007	3.2±0.0173	0.62	99.5
F3	7.0	11.90±0.01	4.11±0.01	0.51	99.67
F4	7.5	11.91±0.0017	4.91±0.032	0.46	100.05
F5	8.2	11.97±0.03	5.16±0.01	0.08	98.82
F6	8.3	11.92±0.02	5.14±0.0057	0.08	99.14
F7	8.3	11.94±0.015	5.18±0.0057	0.075	98.96
F8	8.5	11.90±0.011	5.15±0.01	0.068	99.25
F9	8.7	12.10±0.01	5.16+-0.001	0.06	98.9
F10	10.2	12.1±0.015	5.39+-0.037	0.01	99.9
F11	11.3	12.2±0.009	5.52+-0.001	-	99.82

Physical evaluation of tablets

Table 1 depicts the hardness, diameter, thickness, friability and drug content of various formulations of 5-Fluorouracil. Friability was found to be less than 1%. Hardness of matrix tablets was found to comply with the pharmacopoeial limits.

***In vitro* dissolution studies of matrix tablets**

Dissolution profile of the formulations F1, F2, F3 and F4 in three media of pH 1.2, 6.8 and 6.0 are presented in fig 1. From dissolution studies it was observed that the tablets of batches F1, F2 and F3 released the entire drug within 3 hrs, 4 hrs and 6 hrs of the study respectively but the matrix tablets of batch F4 showed a cumulative percentage release of 69±1.5 and 98±1.47 after 5 hrs and 11 hrs respectively. The hydrophilic nature of pectin may be the reason for the early release of drug from the matrix tablets of formulations F1, F2 and F3. The tablets may swell when they come in contact with aqueous fluids of GIT and cause the release of the entrapped drug through the diffusion and it may not be able to shield the drug load effectively during their passage through the stomach and small intestine.[17] Similar observations have been obtained while evaluating Diltiazem containing pectin matrix tablets coated with inulin and shellac for colon specific drug delivery[16].

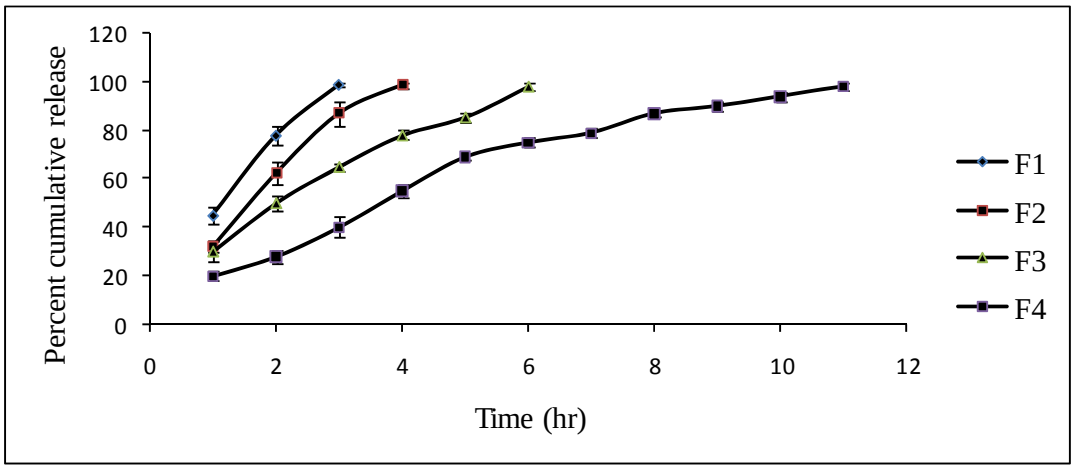

Fig. 1: Plot of cumulative percentage release of 5-Fluorouracil from matrices containing different concentrations of pectin. Vertical bars indicate standard deviation of the means (n=3). Sigma stat software (version 2.03)

Increasing the concentration of pectin in the matrix can protect the drug release for sufficient period of time but in this case initial drug release was still high. So formulation F4 containing 75% w/w of pectin was selected for further coating trials to prevent initial release of drug in stomach and small intestine.

***In vitro* dissolution studies of coated tablets:**

Dissolution profiles of the formulations F4, F6, F7, F8 and F9 in three dissolution media of pH 1.2, 6.8 and 6.0 are presented in fig 2.

The *in vitro* release data of formulations of batch F5 containing 75% w/w pectin coated with Eudragit RS 100 showed a cumulative percent release of 40±5.4% in first 5 hrs and 88±3.21% at the end of 11 hrs. The release of drug in initial 5 hrs was quite high. Therefore in order to further retard the drug release and also to ensure complete drug release in the colonic region, inulin was added due to its degradation specifically in this segment of the GIT by bacteria *bifidobacteria*, which is present only in the colon. Hence, batches F6, F7, F8 and F9 containing 10%, 20%, 30% and 40% of inulin were studied. The decrease in release of drug was observed from batches coated with combination of Eudragit RS100 and inulin as compared to batch F5 that was coated with only Eudragit RS100 (Fig.2).

These results have been supported by IR studies. The spectrum of Eudragit RS100 showed important peak at 1728.10 cm^{-1} due to C=O stretching and spectrum of Inulin showed important band at 3186.16 cm^{-1} -3556.49 cm^{-1} due to O-H stretching. The characteristic bands in case of combination of Inulin and Eudragit RS100 shifted to 1735.81 cm^{-1} (C=O stretching) and 3193.90 cm^{-1} - 3579.64 cm^{-1} (O-H stretching) which indicates the presence of H-bonding between C=O group of Eudragit RS 100 and –OH group of inulin. This H-bonding may be the reason behind the decrease in drug release from batches F6-F9 containing Eudragit RS100 and inulin in different ratios as compared to batch F5 containing only Eudragit RS100 as the coating agent.

Batches F6, F7 and F8 showed a cumulative percent release of 34±3.23%, 26.5±3.62% and 15±2.99% in initial 5 hrs and 79.9±3.14%, 72.1±4.11% and 65.1±4.87% at the end of 11 hrs respectively. Batch F9 containing 40% of inulin in the coating was observed to be the most suitable batch as it showed the drug release of 13.2±3.21% after 5 hrs and 61.2±4.9% after 11 hrs which was less than that released by other formulations. These results have been supported by the fact that as the concentration of inulin is increased in the formulation, inulin gels become more viscous and behave fat-like[29] and this may be the probable reason for the decrease in the release of the drug as the formulation undergoes dissolution in the media.

Despite of coating the formulation F9 with inulin and Eudragit RS100, an initial release of 9.9±1.05% of the drug was obtained in first 2 hrs of dissolution studies, which may be due to the little acid hydrolysis of inulin in 0.1N HCl. It has also been found experimentally that only 10% of inulin is hydrolyzed by acids[21]. Further only 3.3±2.16% release was obtained after 3 hrs of the studies in simulated small intestinal fluid as inulin is found to be resistant to hydrolysis by alkalies[22]. The release of drug in initial 5 hrs can be further retarded by increasing the coat thickness to various levels.

Effect of increasing the coat weight

Dissolution profile of formulations F9, F10 and F11 is shown in the Fig. 3. From release profile it was observed that the drug release from the formulations decreased with the increase in the coat weight. The percent release after 5 hrs followed the order F9> F10>F11. These results indicate that the time required for the release of drug is directly proportional to the coat thickness. The release from the batch F11 was considered to be appropriate as it released only 8.5±2.58% of drug after 5 hrs. This is in accordance with the limits prescribed by USP XXIX, which specifies the release of less than 10% of the incorporated drug for delayed release tablets[23].

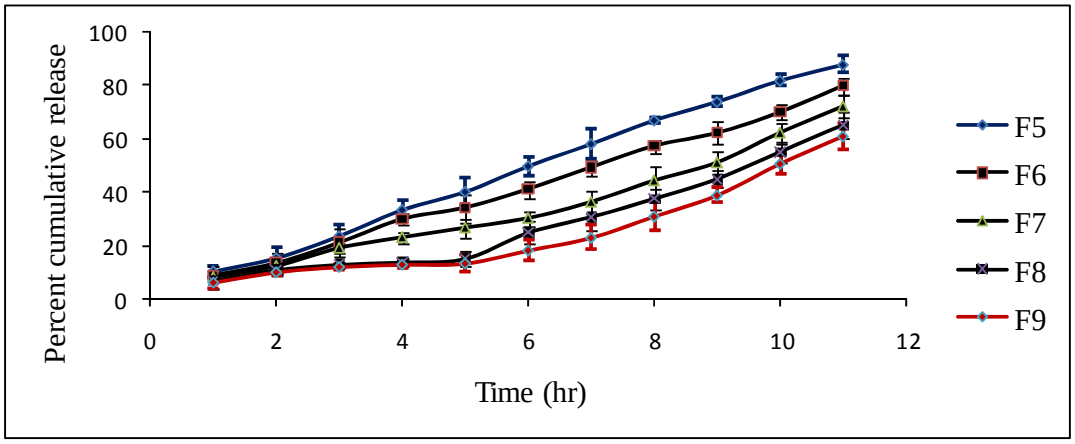

Fig. 2: Plot of cumulative percent release of 5-Fluorouracil from matrices containing 75% w/w pectin coated with combination of Eudragit RS100 and inulin in different ratios. Vertical bars indicate standard deviation of the means (n=3). Sigma stat software (version 2.03)

Though 100% of drug release was not observed in the release studies but complete drug release is proposed in the presence of rat caecal content. Due to the presence of colonic microflora it would aid in degradation of the tablets once the system reaches the colon, thus assisting complete drug release. The complete release of drug further was tested in-vitro in the presence of rat caecal content

Dissolution studies in the presence of rat caecal contents:

The potential use of pectin and inulin as the polymers for colon-specific dosage forms, due to its biodegradability with colonic enzymes has been investigated recently. Earlier studies have shown that pectin[6] and inulin[11] are digested by *Bacteroides* and *Bifidobacteria* respectively, which are present in the colonic microflora. In order to study the susceptibility of the prepared formulation F11 to enzymatic degradation in the presence of colonic bacteria, drug release studies were performed in a similar kind of medium in the presence of 2% w/v caecal content for further 19 hrs after dissolution in simulated gastric and intestinal fluid for 5 hrs Dissolution profile of formulation F11 in the presence and absence of caecal content is shown in Figure 4:

From the *in vitro* release data it was observed that cumulative percent release after 24 hrs increased from 66.1±3.2 % to 87.1±3.55% in the presence of 2% w/v of rat caecal content (Figure 4) as inulin is completely hydrolyzed by some species of colonic bacteria[24]. Considering the fact that the actual concentration of bacteria residing in the human colon is much higher than 2% taken

in the study, complete drug release would occur from the tablets in the human colonic microenvironment.

In-vitro studies in simulated gastric, intestinal and colonic fluids have shown that pectin matrix tablets coated with 12.91% w/w of combination of 60% Eudragit RS100: 40% inulin is capable of protecting the drug from being released completely in the physiological environment of stomach and small intestine and is susceptible to the enzymatic action of the colonic bacteria. Based on these results it was observed that formulation F11 can be successfully used for carrying potent chemotherapeutic agents like 5-Fluorouracil, specifically to the site of action in case of colon cancer. Thus, batch F11 was concluded to be the best formulation as it can ensure the delivery of maximum amount of drug to the colon.

Swelling studies

The swelling behavior of formulations F5, F6, F7, F8 and F9 is shown in Figure 5.

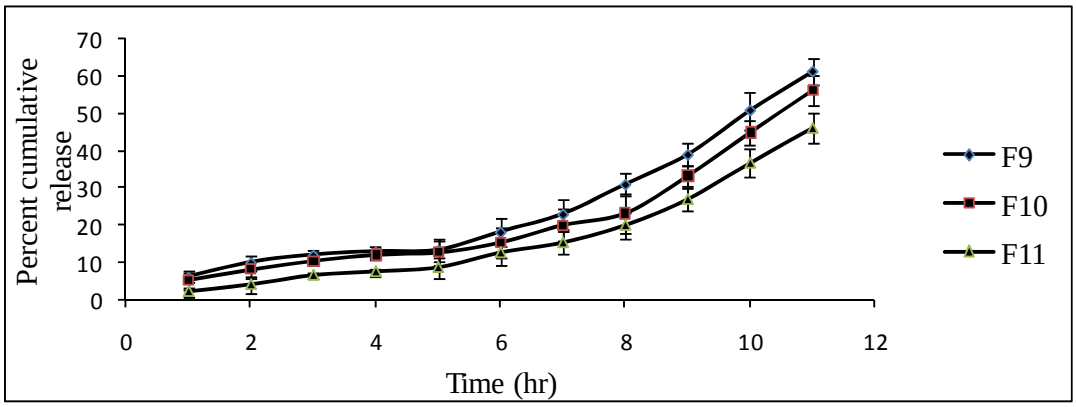

Fig. 3: Plot of cumulative percent release of 5-Fluorouracil from matrices containing 75% w/w pectin coated with combination of 60% Eudragit RS100: 40% inulin at different levels of coating. Vertical bars indicate standard deviation of the means (n=3). Sigma stat software (version 2.03)

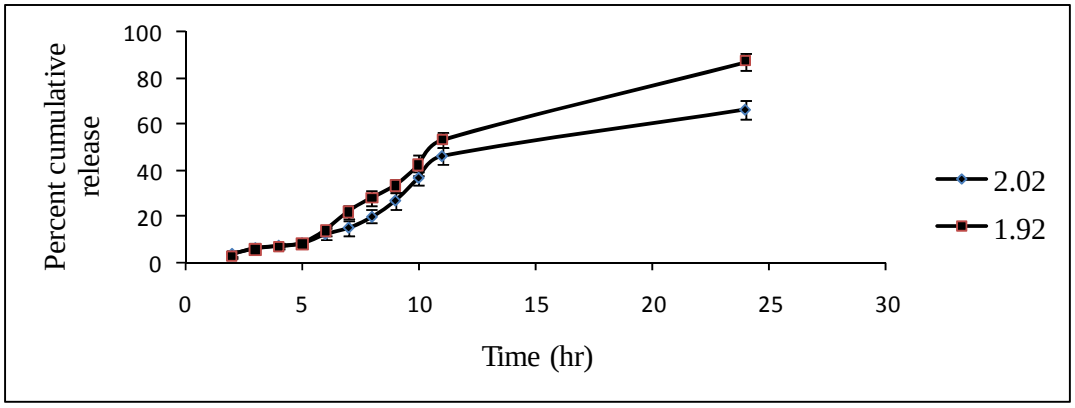

Fig. 4: Plot of cumulative percent release of 5-FU from formulation F11 in the presence and absence of caecal content. Vertical bars indicate standard deviation of the means (n=3). Sigma stat software (version 2.03)

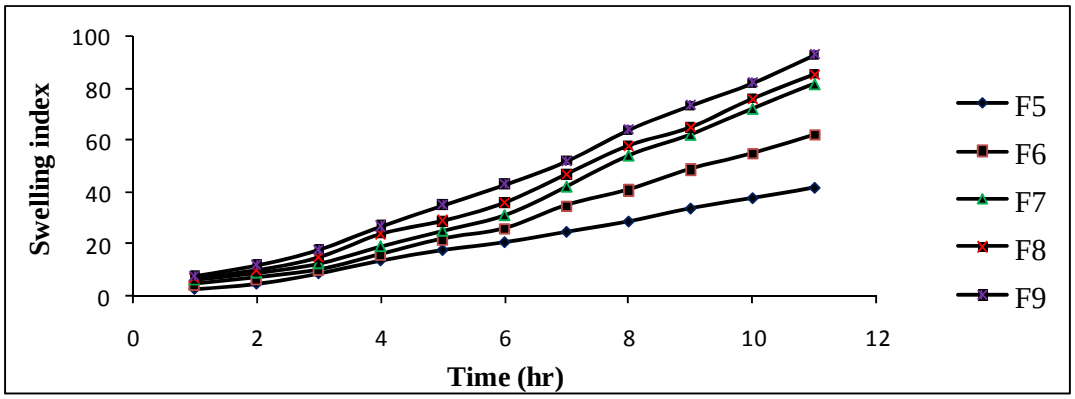

Fig. 5: Swelling behavior showing the swelling indices (%) against time (Batches F5, F6, F7, F8 and F9).

Formulations containing only Eudragit RS100 (F5) had low swelling index in gastric, intestinal and colonic fluid. Eudragit RS 100 has quaternary ammonium groups, which are in the chloride salt form. The dissociation of these quaternary ammonium groups in aqueous media is responsible for the hydration and swelling of this polymer. Indeed, the exchange of chloride ion with the buffer anions of the dissolution medium could govern the degree of hydration and swelling. The gastric media had chloride anion due to hydrochloric acid; which had less selectivity to ion exchange than phosphate and then reduce swelling of polymer in gastric fluid as compared with intestinal fluid[15].

Swelling index of formulations F6, F7, F8 and F9 significantly increased in colonic fluid due to the addition of inulin up to the ratio of 40% which is due to hygroscopic characteristics of inulin as it swells when it comes in contact with dissolution media.

CONCLUSION

The present investigation successfully formulated an oral colon targeted drug delivery system for 5-Fluorouracil. The matrix tablets of 5-Fluorouracil containing different concentrations of pectin, microcrystalline cellulose, magnesium stearate and talc were prepared by direct compression method. The tablets passed all the pharmacopoeial tests. The matrix tablets containing 75% w/w of pectin can protect the drug release for sufficient period of time i.e. it released 98±1.47 % of drug after 11 hrs as compared to the batches containing 30%, 45% and 60% w/w of pectin that released the complete drug within 3 hrs, 4 hrs and 6 hrs of studies respectively. Further this formulation was coated with Eudragit RS100 and inulin in ratios of 100%:0%, 90%: 10%, 80%: 20%, 70%: 30% and 60%: 40% respectively to obtain the weight gain of 9% by weight of tablet weight. From *in vitro* release studies it was concluded that formulation coated with 60% Eudragit RS100: 40% Inulin is the best formulation as only 13.2±3.21 % of drug was released within first 5 hrs which was less than that released by other formulations. Further initial release was retarded by increasing the coating weight and it was concluded that tablets coated with 12.91% w/w of combination of 60% Eudragit RS100: 40% Inulin is an appropriate batch as it released only 8.5±2.58% of drug after 5 hrs which is in accordance with the limits prescribed by USP XXIX. Though 100% of drug release was not observed in the following study, but complete drug release is proposed in the presence of rat caecal content. The release of 87.1±3.55% was observed after 24 hrs in the presence of rat caecal content.

Hence it can be concluded that pectin matrix tablets coated with 12.91% w/w of combination of 60% Eudragit RS100: 40% inulin is capable of protecting the drug from being released completely in the physiological environment of stomach and small intestine and is susceptible to the enzymatic action of the colonic bacteria. Based on these results it was observed that formulation F11 can be successfully used for carrying potent chemotherapeutic agents like 5-Fluorouracil, specifically to the site of action in case of colon cancer. Thus, batch F11 was concluded to be the best formulation as it can ensure the delivery of maximum amount of drug to the colon.

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