BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 49, n. 2, apr./jun., 2013

Article

Formulation, *in vitro* drug release and *in vivo* human X-ray investigation of polysaccharide based drug delivery systems for targeting 5-fluorouracil to the colon

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The purpose of this research study was to develop 5-fluorouracil compression coated tablets by using biodegradable polysaccharide polymer locust bean gum (LBG) and hydroxyl propyl methyl cellulose (HPMC) as coating materials. The fast disintegrating core tablets containing 50 mg of 5-fluorouracil were compression coated with LBG and HPMC in different ratios (8:1, 7:2 and 6:3) with a coat weight of 300, 400 and 500 mg. *In vitro* dissolution data indicated that the formulation (CLH63) with a coat weight of 500 mg containing LBG and HPMC in the ratio 6:3 gave the best release profile (0% in first 5 hour and 96.18% in 24 hours). DSC and FTIR results indicated no possibility of interaction between drug and polymers or other excipients. *In vivo* human X-ray studies revealed that formulation CLH63 was able to resist breakdown in the stomach and small intestine. The disintegration of the tablet occurred in the colon between 8 to 16 hours of post dose. By the present study, it can be concluded that the LBG and HPMC based compression coated tablets of 5-fluorouracil will be useful strategy for colonic delivery of 5-fluorouracil without being released in upper gastrointestinal region for the safe and effective management of colon cancer.

Uniterms: Compression coated tablet. Locust bean gum. Colon targeting. 5-fluorouracil. Colon cancer and X-ray.

O propósito desta pesquisa foi desenvolver comprimidos revestidos de fluoruracila utilizando polissacarídio biodegradável polymer locust bean gum (LBG) e hidroxipropilmetil celulose (HPMC) como materiais de revestimento. Os comprimidos de desintegração rápida contendo 50 mg de fluoruracila foram revestidos por compressão com LBG e HPMC em diferentes proporções (8:1, 7:2 e 6:3), com peso de cobertura de 300, 400 e 500 mg. Os dados da dissolução *in vitro* indicaram que a formulação (CLH63) com peso de cobertura de 500 mg contendo LBG e HPMC na proporção de 6:3 forneceu o melhor perfil de liberação (0% nas primeiras 5 horas e 96,18% em 24 horas). Os resultados de DSC e de FTIR não indicaram interação entre o fármaco e os polímeros ou outros excipientes. Os estudos de raios X *in vivo* revelaram que a formulação CLH63 foi capaz de resistir à quebra no estômago e no intestino delgado. A desintegração do comprimido ocorreu no cólon, entre 8 e 16 horas após a administração da dose. Pelo presente estudo, concluiu-se que os comprimidos de fluoruracila revestidos com LBG e HPMC por compressão se constituirão em estratégia útil na liberação de fluoruracila no cólon, para o tratamento seguro e efetivo do câncer de cólon, sem que o fármaco seja liberado na região gastrointestinal superior.

Unitermos: Comprimido de revestimento por compressão. Locust bean gum. Direcionamento para o cólon. Fluoruracila. Câncer de colon e raios X.

INTRODUCTION

Colon-specific drug delivery systems (CDDS) have attracted a great deal of interest recently for safe and effective therapy for the local treatment of colonic disorders such as Crohn's diseases, infectious diseases, irritable bowel syndrome, ulcerative colitis and colon cancer (Samia *et al*., 2007; Akhgari *et al*., 2009). Drug targeting to colon would also be useful when a delay in drug absorption is desired from therapeutic point of view, such as treatment of diseases that have peak symptoms in the early morning like nocturnal asthma, angina or

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arthritis (Valluri *et al*., 2008). Further it is found to be a promising site for systemic absorption of peptide and proteins. This is because the peptide and protein drugs gets destroyed or inactivated in acidic environment of the stomach or by pancreatic enzymes in the small intestine (Chellan *et al*., 2002). Colonic drug delivery may be achieved by either oral or rectal administration (Mayur *et al*., 2009). Conventional oral dosage forms are ineffective in delivering drugs to the colon due to absorption and /or degradation of the active ingredient in the upper part of the gastrointestinal tract (GIT). Rectal dosage forms (enemas and suppositories) are not always much effective due to high variability in the distribution of drug administered by this route (Sinha *et al*., 2004). Therefore, colon-specific drug delivery systems, which can deliver drugs to the lower gastrointestinal tract without releasing them in the upper part of GI tract, can be expected to decrease the side-effects of the drug and improve the quality of life for the patients suffering from colon-specific diseases (Samia *et al*., 2007).

The various approaches that have been studied for targeting orally administered drugs to the colon include use of pH sensitive polymers, time dependent dosage forms and the use of carriers degraded by the enzymes produced by colonic bacteria (Ashford *et al*, 1993; Rama Prasad *et al*., 1998). Of these approaches, the use of materials that are degraded by the colonic microflora has been found to be the most promising because of their site specificity (Potts *et al*., 1999). Because of the presence of biodegradable enzymes only in the colon, the use of biodegradable polymers for colon specific drug delivery seems to be a more site-specific approach as compared to other approaches. These polymers shield the drug from the environment of stomach and small intestine and are able to deliver the drug to the colon. On reaching the colon, they undergo assimilation by micro-organisms or degradation by enzymes or breakdown of the polymer backbone leading to a subsequent reduction in their molecular weight and thereby loss of mechanical strength. They are then unable to contain drug entity (Huang *et al.,* 1979; Ratner *et al*., 1998; Park, *et al*., 1993).

Complicated by physiological variation in gastrointestinal condition, many Colon specific drug delivery systems (CDDS) designs reported in literature have problems. The goal of CDDS is to cut off precolon drug release and release drug right at the afflicted site. Among the strategies, compression coated systems seem to be superior in preventing premature drug release in stomach and small intestine, while beginning to release the active agents at the proximal colon. On the other hand, the compression coated systems, usually in tablet form, is convenient to manufacture, and no special coating solvents or coating equipment are needed for coating process (Baojain *et al*., 2007).

Colorectal cancer is a common cancer secondary only to lung cancer. 5-Fluorouracil is a pyrimidine analogue and is the drug of choice for colon cancer (Calabresi, Chabner, 1996). Usually it is administered parenterally because absorption after ingestion is unpredictable and incomplete (Hahn *et al*., 1975). Distribution of 5-fluorouracil to undesired sites produces severe toxic effects of the gastrointestinal, hematological, cardiac, neural and dermatological (Diasio, Harris, 1998). Targeting of 5-fuorouracil to the colon for the treatment of colon cancer would not only reduces its systemic toxicity but would also show desired action with a reduced dose. Therefore in the present investigation, based on the physiological characteristics of the GIT, features of enzyme dependent systems and formulation strategies, it could be predicted that such functions into the single delivery systems seems to be desirable to achieve the site-specificity of drug release in the physiological environment of colon. The present research study was proposed to develop a novel biodegradable polysaccharide based compression coated tablet formulations for delivering 5-fuorouracil into the colon by using locust bean gum as a carrier in combination with HPMC and to characterize its colon site-specificity. The developed formulation consisted of two parts, i.e., a fast disintegrating and dissoluting 5-fuorouracil core tablet and outer compression coating layer which could protect or reduce immature drug release in the upper part of gastro intestinal tract and expected to release only in the colon after oral administration due to degradation of the LBG present in the coat by the caecal enzymes action in the colon.

MATERIAL AND METHODS

Material

5-Fluorouracil batch No. 25310FU0108131 (98.0 to 102.0 % pure) was given by Strides Acrolabs ltd., Bangalore, India as a gift sample. Locust bean gum was obtained as a gift sample from lucid colloids Pvt. Ltd., Mumbai, India. Hydroxy propyl methyl cellulose (Colorcon Asia pvt. Ltd. Goa), crosscarmellose sodium and spray dried lactose (Aurobindo pharma, Hyderabad) was obtained as a gift sample. Starch, talc and magnesium stearate (S.D. fine chem. ltd.) were obtained from the local market. All other reagents and chemicals used were of analytical reagent grade.

Methods

Preparation of 5-fluorouracil compression coated tablets

- Preparation of fast disintegrating and dissoluting 5-fluorouracil core tablets

Fast disintegrating and dissoluting 5-fluorouracil core tablets (5-FLU core) were prepared by direct compression formula (Table I) by using spray dried lactose as a direct compression aid and croscarmellose sodium as super disintegrating agent. The 5-fluorouracil, crosscarmellose sodium, lactose, magnesium stearate and talc were accurately weighed and thoroughly mixed with mortar and pestle and passed through the mesh (150 µm) to ensure complete mixing of all the ingredients. The uniformity of mixing was assessed by conducting content uniformity test on the sample of powder mixture. Quantity weighing 100 mg was compressed into tablets using 6 mm round, flat and plain punches on a single station tableting machine (Cadmach, India).

- Preparation of 5-fluorouracil compression coated tablets:

The developed 5-fluorouracil core tablets were compression coated with a coat weight of 500 mg (core coat ratio 1:5), 400 mg (core coat ratio 1:4) and 300 mg (core coat ratio 1:3) containing different ratios of LBG and HPMC. Formulae of different granular coat composition of LBG and HPMC in the ratio 8:1, 7:2 and 6:3 with various excipients were listed in Table II. For compression coating about 45% of coat weight was first placed in the die cavity followed by carefully centering the core tablet and filled with the remainder 55% of coat weight. The coating material was then compressed around the core tablet by using single-station tableting machine with 10 mm plain punches.

- Physical evaluation tests for 5-fluorouracil core and compression coated tablets

Standard physical tests for the developed 5-fluorouracil core and compression-coated tablets were performed and average values calculated. Weight variation was determined by weighing 20 tablets individually, and the average mass and percent variation of each tablet was calculated. Hardness was determined by taking 5 tablets from each batch using a Monsanto hardness tester (Electrolab Pvt. Ltd., India) and the average pressure ($kgcm^{-2}$) applied to crush the tablet was determined. Friability was tested on ten tablets by first weighing and then placing them in a Roche Friabilator, which rotated for 4 min or 100 revolutions. After dusting, the total remaining mass of the tablets was recorded and the percent friability calculated. (Lachman *et al.,* 1987).

Estimation of drug content

The 5-fluorouracil cores as well as compression coated tablets were tested for their drug content to ensure uniformity in drug content. The 10 tablets were finely powdered, and quantity of the powder equivalent to 50 mg of 5-fluorouracil was accurately weighed and transferred to 100 mL volumetric flasks containing 50 mL of phosphate buffer pH 6.8 and allowed to stand for 6 hour with intermittent shaking to ensure complete solubility of the drug. The solution then made up to 100 mL with phosphate buffer pH 6.8 and mixed thoroughly. The solution was filtered, diluted and drug content was estimated by UV-spectrophotometer at the λ_{max} 266 nm (Shimadzu, Japan).

In vitro dissolution studies

- In simulated gastric and intestinal fluid

The ability of LBG polysaccharide based 5-fluorouracil compression coated tablets to protect the drug during the transit time in the gastro intestine region was assessed by mimicking mouth to colon transit. Drug release studies were carried out using USP XXIII dissolution basket method (100 rpm and 37 ± 0.5 °C) in 900 mL pH 1.2 buffer solution for the first 2 hour, as the average gastric emptying time is 2 hour, then the dissolution media is replaced with pH 7.4 phosphate buffer

TABLE I - Composition of fast disintegrating and dissoluting 5-fluorouracil core tablet

S.No.	Ingredients	Category	Quantity/Tablet (in mg)
1.	5-fluorouracil	Active ingredient	50
2.	Croscarmellose sodium	Super disintegrant	05
3.	Spray dried lactose	Direct compression aid	42
4.	Magnesium stearate	Lubricant	02
5.	Talc	Glidant	01
	Total composition	-----	100 mg/tablet

TABLE II - Composition of granular coat formulations for compression coating on 5-fluorouracil core tablets

S.No.	Ingredients	Category	Quantity / Tablet (in mg)								
			ALH81	ALH72	ALH63	BLH81	BLH72	BLH63	CLH81	CLH72	CLH63
1.	Locust Bean gum	Biodegradable polymer	240	210	180	320	280	240	400	350	300
2.	HPMC	Swellable polymer	30	60	90	40	80	120	50	100	150
3.	Starch (Paste)	Binding agent	24	24	24	32	32	32	40	40	40
4.	Magnesium Stearate	Lubricant	3	3	3	4	4	4	5	5	5
5.	Talc	Glidant	3	3	3	4	4	4	5	5	5
	Total coat weight	-----	300	300	300	400	400	400	500	500	500
	LBG : HPMC ratio	-----	8:1	7:2	6:3	8:1	7:2	6:3	8:1	7:2	6:3
	Core : Coat ratio	-----	1:3	1:3	1:3	1:4	1:4	1:4	1:5	1:5	1:5

for 3 hour, as the usual small intestine transit time is 3-5 hour and dissolution was continued in phosphate buffer pH 6.8 up to 24 hour to stimulate the gastrointestinal environment as the usual colon transit time is 20-30 hour. Samples (5 mL) were withdrawn and replaced with fresh medium at fixed time intervals. The sample was suitably diluted and analyzed for percentage of drug release by UV spectrophotometer at the λ_{max} 266 nm.

- In simulated rat caecal content fluid

The susceptibility of locust bean gum coat to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in presence of rat caecal contents because of its similarity to human intestinal microflora (Oluwatoyin, John, 2005). The caecal contents were obtained from male albino rats after pretreatment with 1ml of 2% w/v LBG dispersion in water using gavage for 7 days in order to induce the enzymes acting on polysaccharides. At 45 min before the drug release studies, rats were killed by spinal traction. Their abdomen were opened, the caecum isolated, caecal content removed, weighed and suspended in phosphate buffer (pH 6.8) solution which was previously bubbled with CO_2 to give a final dilution of 4 % w/v, which had been reported to provide the best conditions for assessing the susceptibility to colonic degradation (Prasad *et al*., 1998). Ethical clearance for the handling of experimental animals as per CPSCEA guidelines was obtained from the Institutional Animal Ethical Committee (IAEC) constituted for the purpose. The *in vitro* drug release studies simulating in colon were carried out in USP dissolution rate test apparatus (Apparatus 1, 100 rpm, 37 ± 0.5 °C) with slight modifications as reported earlier by many researchers (Prasad *et al*., 1998). A beaker (capacity 250 mL) containing 200 ml of rat caecal content medium was immersed in the water maintained in 1000 mL vessel, which in turn, was in the water bath of the apparatus. The 5-fluorouracil tablet formulation after completing the dissolution studies in pH 1.2 buffer (2 hour) and phosphate buffer pH 7.4 (3 hour) were placed in the basket of the apparatus and immersed in the phosphate buffer pH 6.8 containing 4% w/v rat caecal conetnt contained in 250 mL beaker and dissolution studies continued up to 24 hour. As the cecum is naturally anaerobic, the experiment was carried out with continuous supply of carbondioxide into the beaker containing rat caecal medium. At the end of the time period 5 mL samples were withdrawn, suitably diluted, centrifuged to remove debris and analyzed for percentage of drug release by UV spectrophotometer at the λ_{max} 266 nm.

In vivo human x-ray studies

X ray imaging was used to monitor the tablets throughout the GI system. Two healthy human volunteers with an age group of 34-36 years and 68-70 kg body weight were recruited in the in vivo studies. Volunteers were non alcoholic, non smoker and had not taken any drugs. The purpose of the study was fully explained and volunteers had given their written consent. After an overnight fast,

subject ingested barium sulfate containing compression coated tablets of optimized batch (based upon *in vitro* drug release studies) orally with 250 mL of water. The tablets were visualized using x-ray. Abdominal radiographs were taken at fixed time intervals (after 2, 5, 8, 12, 16 and 20 hours) and tablets were visualized for site and time range of disintegration of tablet in the colon. The volunteers were served with light breakfast at 2 hour post-dose. This was followed by a standard lunch at 4 hour post-dose. Tea was given at 8 hour post-dose and a standard dinner was given at 12 h post-dose (Demiroz *et al.,* 2004). The experimental protocol and consent for in *vivo* studies were approved by Institute Review Board.

FTIR spectral studies

The KBr pellets with neat drug 5-fluorouracil, powdered tablet formulation of optimized batch CLH63 (selected on the basis of *in vitro* drug release studies) before storage, after storage and placebo tablet formulation of optimized batch were prepared. A FTIR (Shimadzu, FTIR 8400S, Japan) spectrophotometer was used to record IR spectra of the prepared pellets in the range of 400-4000 cm^{-1} with a resolution of 1 cm^{-1} to confirm the absence of chemical interaction of 5-fluorouracil with excipients of core as well as compression coated tablets.

Differential scanning colorimetric studies

The possibility of any interaction between 5-fluorouracil and locust bean gum or other excipients used in the formulation during tablet processing was further assessed by carrying out the thermal analysis on pure drug 5-fluorouracil, powdered tablet formulation of optimized batch CLH63 before storage and after storage at 40 ± 2 °C/75 ± 5% RH for six months by constant rate heating (CRH) method proposed by international confederation for thermal analysis and calorimetry (ICTAC). The DSC curves of the samples were obtained at a standard scanning rate of 10 °C/min conducted over a temperature range of 0 to 350 °C.

Stability Studies

To assess the long term stability of the compression coated tablet formulations, the optimized batch formulation (CLH63) were stored at 40 ± 2 °C/75 ± 5% RH for a period of six months. Samples were observed for any physical change, compressional characteristics and drug content. At the end of six months storage study period, the initial (zero time) results were compared with post-stability testing period results. The powdered samples of 5-fluorouracil compression coated tablet formulations CLH63 were also subjected to DSC and FTIR studies.

RESULTS AND DISCUSSION

The outer coat of LBG and HPMC function as the rate controlling mechanism of 5-fluorouracil release from compression coated tablets, therefore the core tablets were prepared with fast disintegration and dissolution characteristics. Tested in USP disintegration tester (Elico, India), the core tablets were found to disintegrate within 1 min showing required fast disintegration characteristic and over 95% of 5-fluorouracil dissoluted in pH 1.2 buffer within 30 min. The fast disintegration and dissolution of the core tablet prevent it from being the rate limiting factor for release of 5-fluorouracil from compression coated tablets soon after degradation of the biodegradable polysaccharide material locust bean gum content present in the coat by the caecal enzymes in rat caecal medium. The physical property of the 5-fluorouracil core tablet was given in Table III. The core tablet complied with pharmaceutical standards and falls within the limits of Indian pharmacopoeia for weight variation, hardness, friability and drug content uniformity.

All batches (A, B and C series) of compression coated tablets were produced under similar conditions to avoid influence of processing variables. The coated tablets of different formulations were subjected to various evaluation tests, such as hardness, friability, weight variation, drug content uniformity and in vitro dissolution. Physical evaluation results of all the formulations are shown in Table III. In a weight variation test, mass values of the coated tablets were between 1.03% and 2.90%. The average percentage deviation of all tablet formulations was found to be within the limit, and hence all formulations passed the test for uniformity of weight as per official requirements. The hardness of tablets was between 5.92 ± 0.38 and 6.32 ± 0.13 $kgcm^{-2}$. The percentage friability of all the formulations was between 0.238 and 0.447%. Tablets that lose less than 1% of their weight are generally acceptable. In the present study, the percentage friability for all the formulations was below 0.5%, indicating that their friability was within the prescribed limits. Values of the hardness test and percent friability indicate good handling properties of the prepared coated tablets. The drug content uniformity ranged from 98.06 ± 3.02 to 100.03 ± 1.32% which ensures uniformity in drug content in all the coated formulations.

The ability of 5-fluorouracil compression coated tablets to remain intact in the physiological environment

TABLE III - Physical evaluation study for 5-fluorouracil core and compression coated tablets

Formulation	Hardness (kg g cm^{-2} ± SD) (n=5)	Friability (%) (n=10)	Drug Content (% ± SD) (n=10)	Weight Variation (%) (n=20)
5-FLU core	3.10 ± 0.12	0.637	100.89 ± 1.58	2.01
ALH81	5.92 ± 0.38	0.334	99.72 ± 2.82	1.03
ALH72	5.98 ± 0.14	0.285	99.58 ± 1.51	1.46
ALH63	6.04 ± 0.15	0.397	100.03 ± 1.32	2.38
BLH81	5.96 ± 0.27	0.338	98.19 ± 2.72	2.68
BLH72	6.02 ± 0.69	0.238	98.69 ± 2.09	1.98
BLH63	6.22 ± 0.13	0.258	99.27 ± 2.03	1.34
CLH81	6.32 ± 0.13	0.331	98.06 ± 3.02	2.90
CLH72	6.12 ± 0.24	0.447	98.91 ± 1.83	1.60
CLH63	6.06 ± 0.19	0.298	99.23 ± 1.62	2.30
CLH63*	5.64 ± 0.15	0.615	97.75 ± 0.89	1.58

* Data obtained after storage period of 6 months at 40 °C/75% RH.

of stomach and small intestine was assessed by conducting in vitro drug release studies in pH 1.2 buffer for 2 hours, then in phosphate buffer pH 7.4 for 3 hours and continued in phosphate buffer pH 6.8 up to 24 hours without and with rat caecal content in dissolution medium. The results of the *in vitro* drug release studies carried out on 5-fluorouracil core tablets compression coated with a combination of locust bean gum and HPMC in different ratios (8:1, 7:2 and 6:3) and coat weights (300 mg, 400 mg and 500 mg) are shown in Figure 1, 2 and 3.

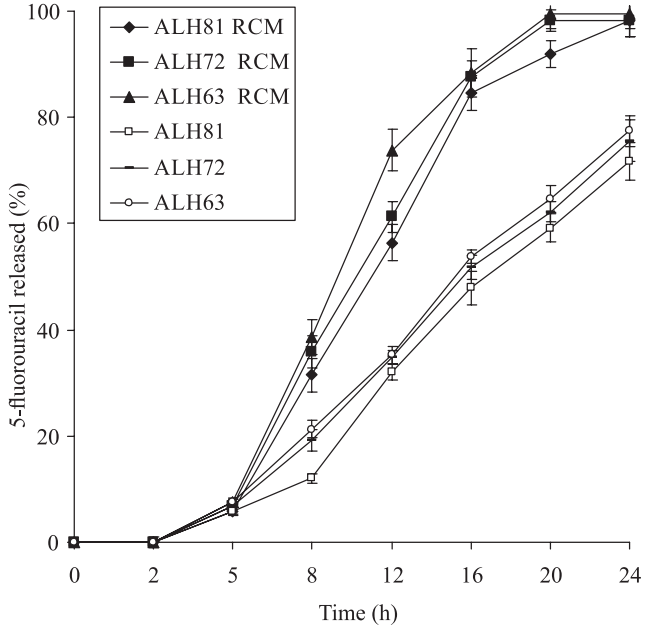

FIGURE 1 - *In vitro* release profile of 5-fluorouracil from formulation coated with 300 mg without and with rat caecal medium (RCM).

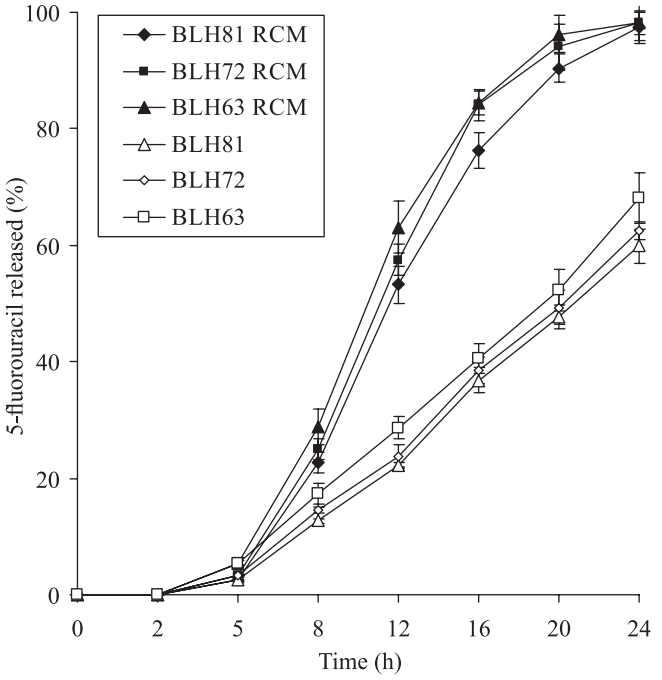

FIGURE 2 - *In vitro* release profile of 5-fluorouracil from formulation coated with 400 mg without and with rat caecal medium (RCM).

The percent 5-fluorouracil released from formulation ALH81, ALH72, ALH63, BLH81, BLH72, BLH63 was 5.85%, 6.92%, 7.52%, 2.59%, 3.36%, 5.42%, respectively, in the first 5 hour of dissolution studies in simulated gastric (2 hours) and intestinal fluid (3 hours) as shown in Figure 1 and 2. From the release profile it was clear that all the formulations with a coat weight of 300 and 400 mg fails to control drug release during the initial 5 h of dissolution studies in simulated gastric (2 hours) and

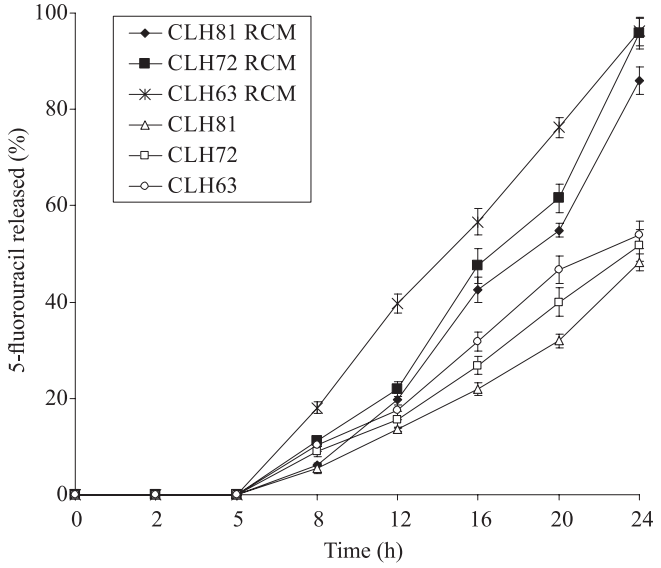

FIGURE 3 - *In vitro* release profile of 5-fluorouracil from formulation coated with 500 mg without and with rat caecal medium (RCM).

intestinal fluid (3 hours). This may be due to the reduced coat weights (300 mg and 400 mg) used around the 5-fluorouracil core tablet, which might not be sufficient to form a thick viscous gel layer around the core tablet to prevent drug release in the first 5 h and were higher as the coat weight decreased around the core tablet. The release of drug ranging from 2.59% to 7.52% in the first 5 hours is a serious consideration for anti cancer drug like 5-fluorouracil which shows deleterious effects on stomach and small intestine.

The oral drug delivery systems targeted to colon should protect the drug being released in the stomach and small intestine. Hence, the coat weight was increased to 500 mg (core coat ratio 1:5) in tablets formulation CLH81, CLH72 and CLH63 as shown in Table II with an objective to prevent the initial premature drug release in upper gastrointestinal region. The formulation CLH81, CLH72 and CLH63 released no drug in the first 5 hours of dissolution study in simulated gastric and intestinal fluid as shown in Figure 3. This suggests that LBG-HPMC mixture with a coat weight of 500 mg can effectively controls the release of the drug in the first 5 hours of dissolution studies. This might be due to high coat weight used around the core tablet and forming a viscous gel layer which retards seeping of dissolution fluid in to the core formulation and controls diffusion of drug from the core into dissolution fluid. It is clearly evident from the *in vitro* release data that on increasing the coat weight around the core tablet minimized the drug release in the initial 5 hours. This might be due to fact that increased coat weights forms a thick viscous gel layer around the core tablet which resulted in an increased resistance of coat to dissolution fluid, causing decreased fluid imbibition consequently causing a decrease in rate of dissolution of drug in core, and ultimately resulted in a decline in drug release.

To assess the integrity of the polysaccharide LBG coat the drug release studies were carried out without addition of rat ceacal content to pH 6.8 phosphate buffer dissolution media. At the end of 24 h of dissolution study, the formulation ALH81, ALH72, ALH63, BLH81, BLH72, BLH63 CLH81, CLH72 and CLH63 were highly swollen and mean percent drug released was 71.76%, 75.60%, 77.41%, 59.84%, 62.47%, 68.13%, 48.22%, 51.72%, 53.94% respectively. It is evident from in vitro release data that, the drug release from all the formulations were found to be incomplete in the physiological environment of colon during the dissolution testing period of 24 hours. This indicates that the locust bean gum present in the coat is not degraded, which was expected to degrade in rat caecal medium, increasing the release of the remaining 5-fluorouracil drug present in the core tablet formulation in to the rat caecal content medium.

The percent 5-fluorouracil released in presence of 4% rat caecal content medium from formulation ALH81, ALH72, ALH63 , BLH81, BLH72, BLH63, CLH81, CLH72 and CLH63 was found to be 98.16%, 98.27%, 99.51% 97.52%, 98.16%, 98.34%, 85.94%, 95.73% and 96.18%, respectively, at the end of 24 hour as shown in Figure 1, 2 and 3. The release profiles revealed that drug release was increased rapidly and more then 95% of the drug was released from all the formulations except CLH81 in rat caecal medium compared with that in with out rat caecal content medium. The formulation CLH81 released only 85.94% of the drug, this is due to high proportion of LBG content present in the coat shell which might have not disintegrated completely during the dissolution testing period of 24 hours in rat caecal medium. The increase in the release rate in rat caecal medium was most probably due to biodegradation of LBG present in the coat by colonic bacterial enzymes. It is evident from the Figure 1, 2 and 3 that the coat weights used for coating around the core tablet had a marked effect on drug release rate in the rat caecal content medium. As the coat weight decreases, rate of degradation of LBG in rat caecal medium increases, which caused increment of drug release from formulations. It could be seen that release rate was higher in formulation of A and B series compared with that in formulation of C series, where proportion of LBG or coat weight was higher. The rate of drug release was found to increase from 8 h in all the formulations with a coat weight of 300 and 400 mg and in formulations with a higher coat weight of 500 mg

the release rate was found to increase from 12 hours. The in vitro results revealed that for protecting 5-fluorouracil core tablet in the physiological environment of stomach and small intestine, the 5-fluorouracil core tablet should be coated with a higher coat weight (500 mg).

In vitro release studies in the presence of rat caecal content indicated the suitability of the polysaccharide locust bean gum based compression coated tablets for colonic delivery of 5-fluorouracil in case of LBG and HPMC mixture in the ratio 6:3 with a coat weight of 500 mg as coating materials. However, the evaluation of the dosage form in human renders support to *in vitro* studies. Hence, X-ray studies were carried out on healthy human volunteer to access the *in vivo* performance and to support in vitro drug release studies of the optimized batch formulation CLH63. The studies were carried out using barium sulphate as X-ray opaque material. As per the advice of a well-known radiologist of the city, the time and amount of radiation exposed to volunteers would be more if γ-scintigraphy technique is adopted. Also γ-scintigraphy technique utilizes the radiotracers, which may harm the volunteers. Further, X-ray visualization of tablet disintegration performance has been studied by many researchers (Munira, Pundarikakshudu, 2007). Hence, it was proposed to use roentegenography technique which is comparatively safer technique. The optimized batch compression coated tablet formulation CLH63 containing barium sulphate was ingested by the health volunteers and images were taken at different time intervals. The position of the test tablet formulation throughout GI system at different time points were shown in Figure 4.

From the abdominal radiographs taken at different time intervals, it is evident that tablet remained unchanged form in the stomach and after 2 hour it is found in the jejunum as shown in Figure 4 (A). After 5 hours, the tablet appeared to be slightly swollen but remained intact and reached the ileocecal region without disintegrating in the upper region of GI tract this is due to the resistance of the coat to the stomach and intestinal fluid. The X-ray images showed that the tablets slowly disintegrated in the colon (ascending colon/hepatic flexure) after reaching colon between 5 and 8 hours of post administration. It is evidenced by reduction in swollen tablet size appeared in X-ray images taken after 8 hours, 12 hours and 16 hours. This can be attributed to a possible degradation of locust bean gum and solubilization of HPMC present in the coat in the physiological environment of colon. Complete disintegration of the tablet was seen clear after 20 hours post administration. This was attributed by the disappearance of the tablet as shown in X-ray image Figure 4 (F). These results are in agreement with the results of Ashford *et al.* (1993) who observed that the gastric emptying time of 0.6-2.9 hour, small intestine transit time of 1.8-8.5 hours and colonic arrival time of 3.2-9.8 hours while evaluating pectin as a compression coat for colonic drug delivery, using gamma scintigraphy.

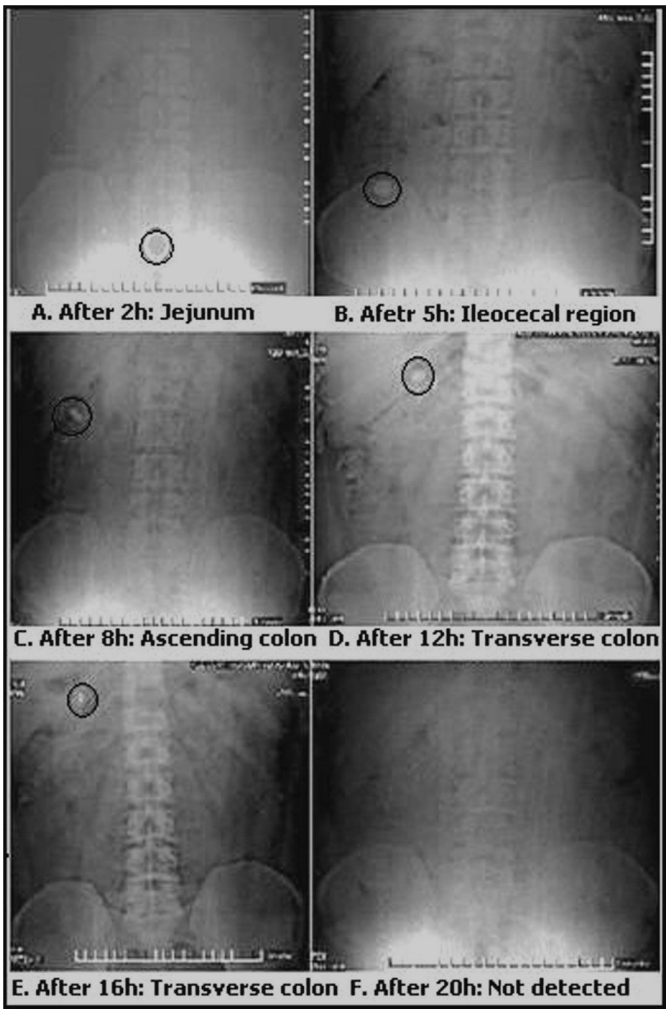

FIGURE 4 - The X-ray images at different time points showing tablet formulation CLH63 localization in the gastrointestinal tract.

The FTIR spectrum of 5-fluorouracil (A), formulation CLH63 before storage (B) and formulation CLH63 after storage (C) and placebo formulation of CLH63 (D) are represented in Figure 5. In case of FTIR spectra of 5-fluororuacil, bands at 3053 cm^{-1}, 3014 cm^{-1} and 2825 cm^{-1} are attributed to both aromatic and aliphatic C−H stretching vibrations. A band at 1726 cm^{-1} represents the imide group stretching of heterocyclic ring. A band at 1661 cm^{-1} is due to the tertiary amide group stretching vibration. N−H bending vibration is observed at 1517 cm^{-1}. A band at 1236 cm^{-1} shows C−N stretching vibrations.

The C-F stretching band was observed at 806 cm^{-1}. When FTIR spectra of 5-fluorouracil were compared with FTIR spectra of compression coated tablet formulation CLH63 (before storage and after storage) there was no change in the position of the principal peaks/bands of 5-fluorouracil. This further confirms the integrity of pure drug 5-fluorouracil and their compatibility with the excipients of core as well as compression coated tablets.

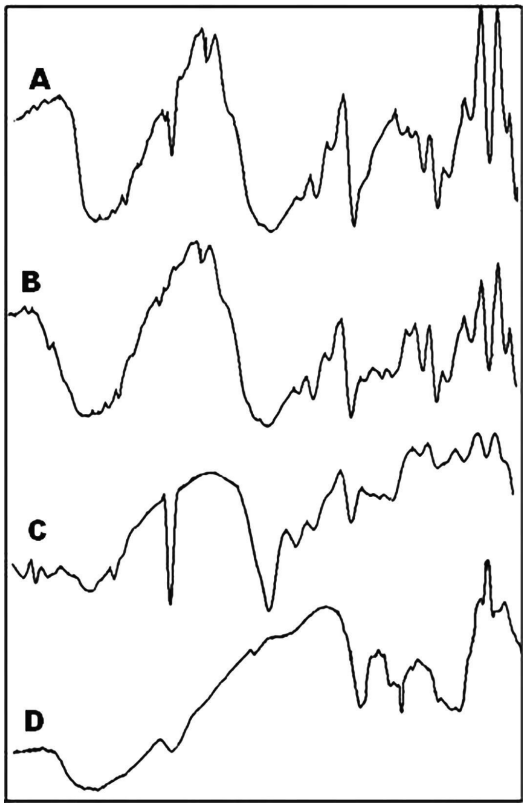

FIGURE 5 - FTIR spectra of 5-fluorouracil (A), formulation CLH63 before storage (B), formulation CLH63 after storage (C) and placebo formulation of CLH63 (D).

DSC curves of 5-fluorouracil (A), formulation CLH63 before storage (B) and formulation CLH63 after storage (C) and placebo formulation of CLH63 (D) obtained by constant rate heating (CRH) / constant rate thermal analysis (CRTA) method proposed by international confederation for thermal analysis and calorimetry (ICTAC) are shown in Figure 6. DSC curve of 5-fluorouracil showed a single sharp endothermic peak at 284.5 °C, which was ascribed to drug melting. The endothermic peak corresponding to melting point of 5-fluorouracil in formulation CLH63 before storage did not show any significant shift in the endothermic peak 285.68°C. The DSC curve of formulation CLH63 after storing at 40 ºC/75 % RH for 6 months, the endothermic peak was slightly shifted to 275.17 °C. The findings of the DSC study further confirmed absences of interaction between 5-fluorouracil and excipients used in the core as well as compression coated tablets.

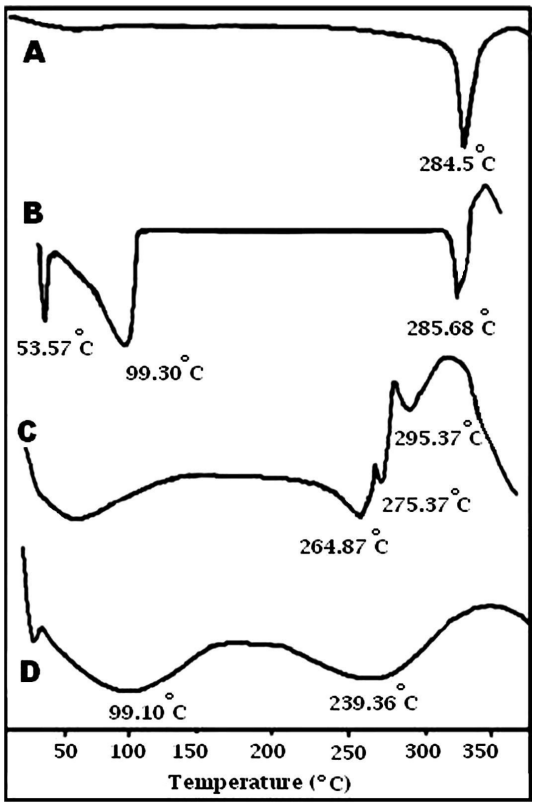

FIGURE 6 - DSC curves of 5-fluorouracil (A), formulation CLH63 before storage (B), formulation CLH63 after storage (C) and placebo formulation of CLH63 (D).

It was observed that, there was no significant change in hardness, friability, weight variation and drug content uniformity of the optimized batch formulation CLH63 after storage at 40 °C/75% RH for 6 months as shown in Table III. Figure 7 represents the in vitro release profile of 5-fluorouracil from optimized tablet batch formulation CLH63 after storage period of six months and there was no significant difference in the percent 5-fluorouracil released from the same formulation CLH63 before storage. This indicates that the formulation CLH63 could provide a minimum shelf life of 2 years (Mathews, 1999).

CONCLUSION

From the results of *in vitro* dissolution profile, we are able to conclude that fast disintegrating 5-fluorouracil core tablet compression coated with a coat formulation CLH63 found to be suitable for successful colonic delivery

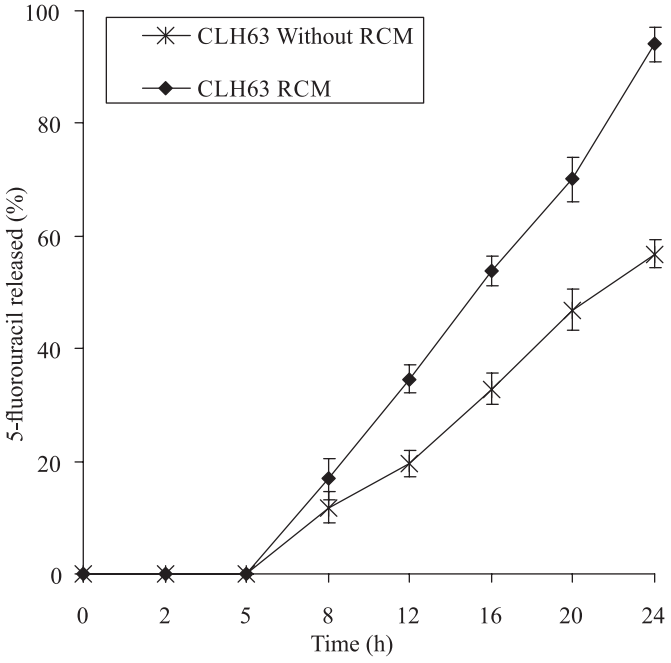

FIGURE 7 - Release profile of 5-fluorouracil from formulation CLH63 after storage at 40 °C/75% RH for 6 months.

of 5-fluorouracil without being released in physiological environment of stomach and small intestine. *In vivo* X-ray studies using healthy human volunteers were also performed in which coat formulation CLH63 showed disintegration of the tablet in the ascending colon between 8 to 16 hours of post dose. Therefore, this study lays a basis for use of polysaccharide LBG along with HPMC for compression coating as one of the novel approaches for colonic delivery of 5-fluorouracil for the treatment of colon cancer.

ACKNOWLEDGEMENTS

Author is thankful to the Management, K.R.E.society's Karnataka College of pharmacy, Bidar, Karnataka (India) for extending research facility to carryout this research work. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Received for publication on 02nd March 2012
Accepted for publication on 03rd January 2013