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Research Article

BORASSUS FLABELLIFER FRUIT MUCILAGE: POTENTIAL BIODEGRADABLE CARRIER FOR COLON SPECIFIC DRUG DELIVERY

RAVI KUMAR*[1], RAJARAJESHWARI N[2], NARAYANA SWAMY VB[3]

[1]Research Scholar, Shri Jagdish Prasad Jhabarmal Tibrewala University, Rajasthan, [2]Visveswarapura Institute of Pharmaceutical Sciences, Bangalore, [3]Karavali College of Pharmacy, Vamanjoor, Mangalore. Email: ravikumar300@gmail.com.

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ABSTRACT

The present study was undertaken to assess the potential of Borassus flabellifer mucilage (BFM) to act as a biodegradable carrier for colon specific drug delivery. Hence an attempt was made to develop matrix tablet of olsalazine sodium based formulation using BFM which protects the drug in upper GIT and release the major amount of drug in colon due to degradation by bacterial enzymes. Colon targeted matrix tablets of olsalazine sodium were prepared by direct compression method using different concentrations viz; 5, 10, 15, 20 and 25% w/w of BFM. Tablets were evaluated for hardness, friability, weight variation, drug content, in vitro dissolution test in simulated gastric, intestinal and colonic fluid with and without 4% (w/v) rat caecal matter and stability study. FT-IR and DSC studies confirmed the absence of any drug polymer interaction. In vitro studies revealed that the tablets containing 25%w/w of BFM (F5) have limited the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment. Optimized formulation (F5) showed no change either in physical appearance, drug content or in dissolution pattern after storage at 400C/RH75% for 3months. Mathematical modeling showed that release pattern follows the Peppas model. X-ray images were taken to investigate the movement, location and the integrity of the tablets in different parts of gastro intestinal tract in rabbits and it clearly supported the in vitro study. The study showed that BFM can be used successfully for colon specific drug delivery system.

Keywords: Colon targeting, *Borassus flabellifer* mucilage, *In vitro* dissolution, Rat caecal matter, gastrointestinal tract.

INTRODUCTION

Colonic drug delivery has gained increased importance not just for the delivery of the drugs for the treatment of local diseases associated with the colon like Crohn's disease, ulcerative colitis, irritable bowel syndrome but also for the potential it holds for the systemic delivery of proteins and therapeutic peptides. The large intestine, though difficult to reach by peroral delivery, is still deemed to be the ideal site for the delivery of agents to cure the local diseases of the colon[1-2]. The most critical challenge in such drug delivery approach is to preserve the formulation during its passage through the stomach and about first six meters of the small intestine[3]. In order to develop a reliable colonic drug delivery system, the transit time of dosage forms through the gastrointestinal (GI) tract needs to be understood very well. The transit of perorally administered formulation through the GI tract is highly variable and depends on various factors[4-9]. Due to the distal location of the colon in the GI tract, a colon specific drug delivery system should prevent drug release in the stomach and small intestine, and affect an abrupt onset of drug release upon entry into the colon. Such a system can be formulated utilizing some specific conditions existing in the colon in comparison to other parts of the GI tract. Overall, the physiological changes along the GI tract can be generally characterized as a continuum, with decrease in enzymatic activity, motility and fluid content and an increase in pH as we move from esophageal end to the rectum. Another challenge in developing therapeutically effective products for the treatment of colonic pathologies is the impact of disease on the delivery system[10]. In general four primary approaches have been proposed for colon targeted delivery namely prodrugs, pH dependent system, time dependent systems and colonic micro flora activated systems. Among the different approaches to achieve colon specific drug delivery, the use of polymers, specifically biodegraded by colonic bacterial enzymes holds promise[11-12]. The important bacteria present in the colon such as Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Lactobacillus, Clostridium secrete a wide range of reductive and hydrolytic enzymes such as β-glucuronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase. These enzymes are responsible for degradation of di-, tri- polysaccharides[13-14].

Polymers have been successfully investigated and employed in the formulation of solid, liquid and semi-solid dosage forms and are specifically useful in the design of novel drug delivery systems. Both synthetic and natural polymers have been investigated extensively for this purpose. Synthetic polymers are toxic, expensive, have environment related issues, need long development time for synthesis and are freely available in comparison to naturally available polymers[15]. However the use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available, non-toxic and capable of chemical modifications, potentially biodegradable and with few exceptions and also biocompatible.

A large number of plant-based pharmaceutical excipients are available today. Many researchers have explored the usefulness of plant-based materials as pharmaceutical excipients. Ability to produce a wide range of material based on their properties and molecular weight, natural polymers became a thrust area in majority of investigations in drug delivery systems[16]. Natural gums can also be modified to meet the requirements of drug delivery systems and thus can compete with the synthetic excipients available in the market[17].

The *Borassus flabellifer* is a tall and erect palm, with large, fan-shaped leaves which are quite unlike the pinnate leaves of other palms. The different parts of the plant is used for the various ailments like secondary syphilis, antiperiodic, heart burns, liver and spleen enlargement etc. Other than these pharmacological uses the juice of the plant is used in preparation of health drinks, jellies etc. The leaves are use to make baskets, hats and many other useful items. Borassus flabellifer contains albuminoids, fats and the fresh pulp is reportedly rich in vitamins A and C. The fresh sap is reportedly a good source of vitamin B-complex. Male inflorescence constitutes spirostane-type steroid saponins like borassosides and dioscin. It also contains 20 known steroidal glycosides and carbohydrates like sucrose. It also contains bitter compound called flabelliferrins, these are steroidal saponins[18-19]. The endosperm contains a high proportion of mucilage. The two major polysaccharides present in this endosperm are galactomannan and mannan.

During earlier study in our laboratory, the disintegrating, binding, gelling, release retardant and suspending properties of *Borassus flabellifer* mucilage (BFM) were evaluated. Literature survey reveals that comprehensive physicochemical characterization and pharmaceutical application of the BFM as carrier for colonic drug delivery has not been reported yet. Hence the present work was

attempted to evaluate the potential of BFM to act as a biodegradable carrier for colon specific drug delivery using olsalazine sodium as a model drug.

MATERIALS AND METHODS

Materials

Olsalazine sodium was obtained from Karnataka Chemsyn Limited, Bangalore, India as gift sample. Microcrystalline cellulose, talc and magnesium stearate were obtained from Zydus Research centre, Ahmadabad, India as gift samples. *Borassus flabellifer* endosperm was procured from the local market of Udupi district, Karnataka. All the other solvents, reagents and chemicals used were of either Pharmacopoeial or analytical grade.

Methods

Isolation and purification of mucilage from *Borassus flabellifer* endosperm[20]

The endosperm of *Borassus flabellifer* fruit contains mucilage. To increase the yield of the mucilage the endosperm of *Borassus flabellifer* fruit were extracted by different solvents. The endosperm of *Borassus flabellifer* were collected, cut into small pieces and dried using tray dryer at 37°C for 24 h at room temperature, made fine powder by crushing in a mixer. The fine powder was soaked in different solvents such as water, hot-water, phosphate buffer solution (PBS) of pH 4.0, 6.8, 9.2, separately for 2-3h and heated up to 80-90°C for 30-45 min for complete release of the water soluble mucilage into the solvents. The mucilage was then extracted by using a multi layer muslin/cheese cloth bag to remove the marc and concentrated viscous solution under reduced pressure at 60-70°C. Acidified ethanol (5% HCl in 75% ethanol) was added to the concentrated viscous solution with constant stirring. The gel like precipitate was formed and separated by filtration. The precipitate was washed 2-3 times with 75% and 95% ethanol. After complete washing of the precipitate with ethanol 95%, brownish white powder was obtained. The powder was dried in an oven at 37°C, collected, grounded, passed through a # 80 sieve and stored in a desiccator till use. The brownish white powder was considered as mucilage for pharmaceutical use. Physicochemical characterization, phytochemical screening and toxicity studies of the isolated mucilage were carried out as per the reported procedure[21-24].

Drug-Excipient Compatibility study

This study has been done to check whether there is any compatibility related problems are associated with drug and excipients used for the formulation of tablet.

Fourier Transform Infrared (FTIR) Spectral analysis

FTIR spectra of pure drug and physical mixture of drug and excipients were recorded on samples prepared in potassium bromide (KBr) disks using a FTIR spectrophotometer (FTIR-8300, Shimadzu, Japan). Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 400 to 4000 cm^{-1}.

Differential Scanning Calorimetry (DSC) analysis

DSC analysis was performed using Shimadzu DSC-60, Shimadzu Limited Japan. A 1:1 ratio of drug and excipient was weighed into aluminum crucible, and sample was analyzed by heating at a scanning rate of 20^0C over a temperature range 40-300^0C under nitrogen environment.

Biodegradation studies of BFM

The *in vitro* biodegradation studies of BFM were carried out in presence and absence of 2% w/v and 4% w/v rat caecal contents (RCC) before and after enzyme induction by viscosity measurement by using 1% w/v dispersion of BFM after incubation at 37°C for 24 h.

Formulation of colon targeted tablets of olsalazine sodium

The Colon specific tablets were prepared by direct compression method. Olsalazine sodium was used as a model drug. Five formulations were prepared using different concentrations of BFM (5% to 25%w/w) as shown in table 1. All the tablets contained 100 mg of olsalazine sodium, mixed properly with BFM, microcrystalline cellulose and other excipients like talc and magnesium stearate, slugged and these powder blends were evaluated for pre-compression characteristics prior to compression. After determining precompression characteristics powder blends were compressed in 12 station rotary tablet Mini press–I with 8mm concave faced punches (Cemach, Ahmadabad, India) to obtain 400 mg olsalazine sodium containing BFM tablets.

Table 1: Formulation of colon targeted olsalazine sodium tablets

Ingredients*	Formulation code				
	F1	F2	F3	F4	F5
Olsalazine sodium	100	100	100	100	100
*BFM	20	40	60	80	100
Microcrystalline cellulose	271	251	231	211	191
Magnesium stearate (1%)	3	3	3	3	3
Talc (2%)	6	6	6	6	6
Total weight of tablet	400	400	400	400	400

BFM* = *Borassus flabellifer* mucilage; All the quantities are in mg

Evaluation of precompression characteristics of olsalazine sodium powder blend

The prepared olsalazine sodium powder blends were evaluated for angle of repose, tapped, bulk density, compressibility index and Hausner's ratio as per the reported methods.

Evaluation of olsalazine sodium colon targeted tablets[25-28]

The following evaluation tests were carried out on formulated tablets which includes;

General appearance

Two tablets from each formulation were randomly selected and organoleptic properties such as colour, odour, taste, and shape were evaluated.

Weight variation test

Randomly twenty tablets were selected after compression, weighed individually and average weight was determined.

Hardness Test

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The crushing strength of the tablets was measured using a Monsanto hardness tester. It is expressed in kg/cm^2. Five tablets from each formulation batch were tested randomly and the average reading noted.

Friability Test

The friability of tablets was determined using Roche Friabilator. It is expressed in percentage (%). Ten tablets were initially weighed (W_0) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W). The % friability was then calculated by,

$$\%F = 100\ (1\text{-}W_0/W)$$

Drug content uniformity

The olsalazine sodium tablets were tested for their drug content uniformity. At random 20 tablets were weighed and powdered. The

powder equivalent to unit dose of tablet was weighed accurately and mixed in 100ml of phosphate buffer pH 6.8. The mixture was shaken properly. The un-dissolved matter was removed by filtration through Whatman No.41 filter paper. The Absorbance of the solution was measured at 245 nm. The concentration of the drug was computed from the standard curve of the olsalazine sodium in phosphate buffer pH 6.8.

Thickness and diameter

The thickness and diameter of the tablets was determined by using vernier calipers. Five tablets were used and average values were calculated.

Swelling index

The extent of swelling was measured in terms of % weight gain by the tablet. The swelling behavior of all formulation was studied. One tablet from each formulation was randomly selected, weighed individually(W_1) and placed separately in a wire basket which was placed in a 100 ml beaker containing 0.1 N HCL for first 2h and in pH 6.8 phosphate buffer for remaining 22h. At the end of 2, 4, 6, 8 and 24 hrs tablets were withdrawn from wire basket and excess water was removed using tissue paper. The swollen tablets were reweighed (W_2) and swelling index of each tablet was calculated by using the formula;

$$\text{Swelling index (\%)} = \frac{W_2 - W_1}{W_1} \times 100\%$$

The experiment was performed in triplicate for each time point and fresh samples were used for individual time point.

Disintegration test

The disintegration time of tablet was determined by placing one tablet in each of the six tubes of the basket and operated the apparatus, using pH 1.2 buffer solution maintained at 37±0.5°C. Then the disintegration time of tablet is recorded. The experiment was repeated for three times and average was noted.

***In vitro* drug release studies**

The ability of olsalazine sodium tablet to remain intact in the physiological environment of stomach and small intestine was assayed by mimicking mouth to colon transit. The dissolution studies were carried out in 0.1N HCl (SGF) for first 2 h (as the average gastric transit time is (~ 2 h), then in 7.4 pH Phosphate buffer(SIF) for next 3 h (as average intestinal transit time) and for remaining hours in simulated colonic fluid (SCF) prepared in pH 6.8 saline phosphate buffer to mimic colonic pH. Drug release studies were carried out using USP (23) dissolution apparatus II (paddle). The apparatus was maintained at 37±0.5˚C and at 100 revolutions per minute. The height of paddle was adjusted at about 2cm above the bottom surface. About1 ml of sample was withdrawn at regular interval of time and diluted to 10 ml in volumetric flask. Finally the drug concentrations in samples were measured in triplicates using UV-spectrophotometer (Shimadzu-UV1601 spectrometer, Japan) at 245 nm.

***In vitro* drug release study with 4% (w/v) rat caecal matter**

In order to assess the susceptibility of mucilage being acted upon by colonic bacteria, drug release studies were also carried out in presence of rat caecal content because of the similarity of human and rodent colonic micro flora. On the basis of performance of *in vitro* dissolution study in SGF, SIF and SCF for 24 h, the best one formulation which gave lowest cumulative release in SGF and SIF, but highest cumulative release in SCF was selected for further *in vitro* dissolution study with 4% (w/v) rat caecal matter. Initial studies were carried out at pH 1.2 for 2 h, after this, the dissolution medium was changed to pH 7.4 for 3 h followed by pH 6.8 containing 4% (w/v) rat caecal matter and the dissolution was continued until the completion of 24 h. The dissolution was performed in six stages USP (23) dissolution apparatus (paddle type), but slight modification was made to it. A beaker (capacity 250 ml, internal diameter 55 mm) containing 100 ml of dissolution medium was immersed in the water containing in 1000 ml dissolution flask, which was in turn, in the water bath of the apparatus. The tablets were placed in the dissolution apparatus and immersed in the dissolution medium containing 4% (w/v) caecal contents. The sample were withdrawn at regular intervals without pre-filter and replaced with fresh buffer. Absorbance of the sample was measured in UV spectrophotometer at absorption maxima of the drug and concentration was calculated by regression equation. The experiments were carried out with continuous CO_2 supply into the beakers to simulate anaerobic environment of the caecum.

Mathematical treatment of the *in vitro* release kinetics

To examine the drug release kinetics and mechanism, the release kinetics of the best one formulation was analyzed according to zero order, first order kinetics, Higuchi and Korsmeyer-Peppas model. The correlation coefficients (r^2) were calculated for linearity.

Scanning Electron Microscopy studies (SEM)

In order to elucidate the mechanism of drug release from optimized formulation F5, surface of optimized tablet, both before and after dissolution studies, were studied using scanning electron microscope (SEM). The samples were placed on a spherical brass stub (12 mm diameter) with a double backed adhesive tape. The Tablets (before dissolution studies) were mounted as such on the specimen stub. On the other hand, small sample of the coating membrane was carefully cut from the exhausted shells (after 24 h of dissolution studies) and dried at 50°C for 6 h. The mounted samples were sputter coated for 5 to 10 min with gold using fine coat ion sputter and examined under SEM (JEOL, JSM-6100, Japan).

Stability study

Accelerated stability study of the optimized formulation was conducted according to ICH guidelines by subjecting to stability testing at 40°C± 2°C, 75% RH for 90 days. At the end of 90 days period, tablets were evaluated for physical appearance, drug content and *in vitro* release pattern.

***In vivo* targeting efficiency**

The evaluation of dosage form in animal model renders support to the *in vitro* studies. To closely simulate the human physiological environment of the colon, rabbits were selected as animal model after obtaining ethical clearance for evaluating the colon specific delivery (Approval No:KCP/IAEC/Ph.Ceutics/05/2011-2012). In this study, healthy rabbits were fasted overnight. Roentgenography study; a comparatively safer technique was carried out in healthy male albino rabbits to access the *in vivo* performance of the selected batch. The study was carried out using barium sulphate as X-ray opaque material. Tablets containing barium sulphate (15%) for the selected batch was formulated and administered to rabbits with a glass of water. After the administration of the formulation, X-ray images were taken under the supervision of a radiologist, to follow the movement, location and the integrity of the tablets in different parts of GIT.

RESULTS AND DISCUSSION

Oral drug delivery represents one of the frontier areas of controlled drug delivery system. Colon targeted drug delivery system belongs to oral drug delivery system group, which is capable of protecting the release of the drug in the stomach and small intestine and release the drug in the colon.

Drug-Excipients Compatibility Studies

Fourier transform infrared (FTIR) analysis

FTIR spectra were recorded to assess the compatibility of the drugs and excipients. FTIR spectra of drug (s), physical mixture of drug with different excipients, were recorded and examined. FTIR spectra of olsalazine sodium showed principal peaks at 1710 and 1740cm^{-1} resulted from C=O stretching and the peak at 2900cm^{-1} resulted from Carboxylic O-H stretching and peaks at 3350, 3020 and 1552 cm^{-1} resulted from aromatic O-H stretching, aromatic C-H stretching and aromatic C=C stretching respectively. The observed FTIR spectrum of drug was matched with reference spectra. Confirming

the purity of the drug as per established standards. All characteristic peaks of drug(s) were observed in the FTIR spectra of physical mixture of drug and different excipients. The results showed there was no appearance or disappearance of peaks in the polymer–drug mixture this confirmed the absence of any chemical interaction between the drug and the polymers. The FTIR spectra of pure drug and physical mixture of drug and different excipients are shown in figure1 and 2 respectively.

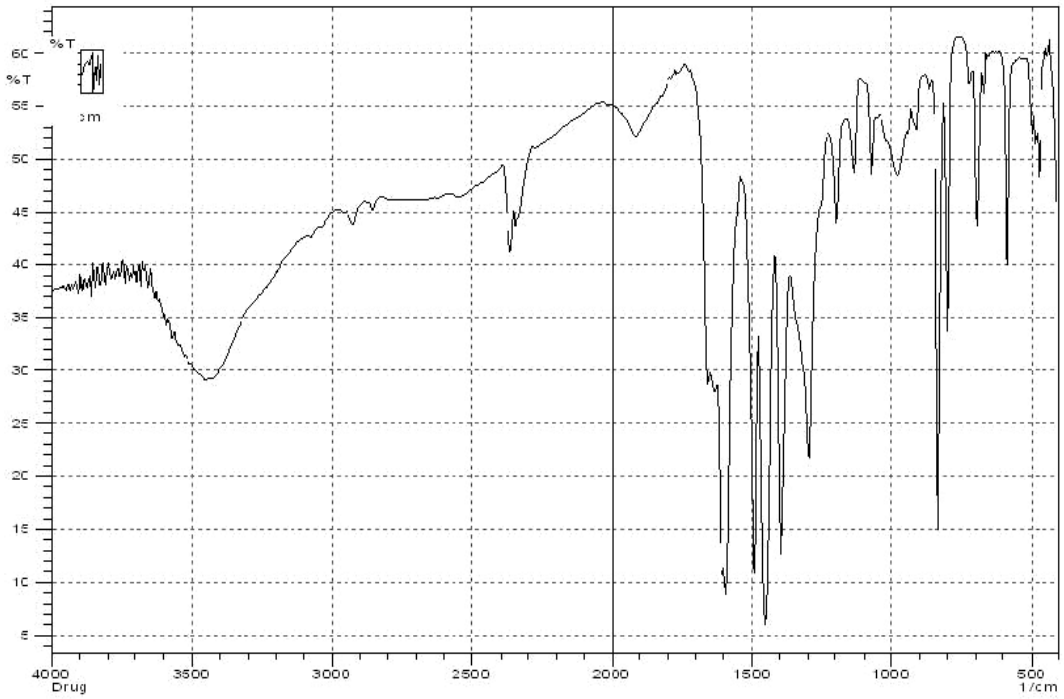


Fig. 1: FTIR Spectrum of olsalazine sodium

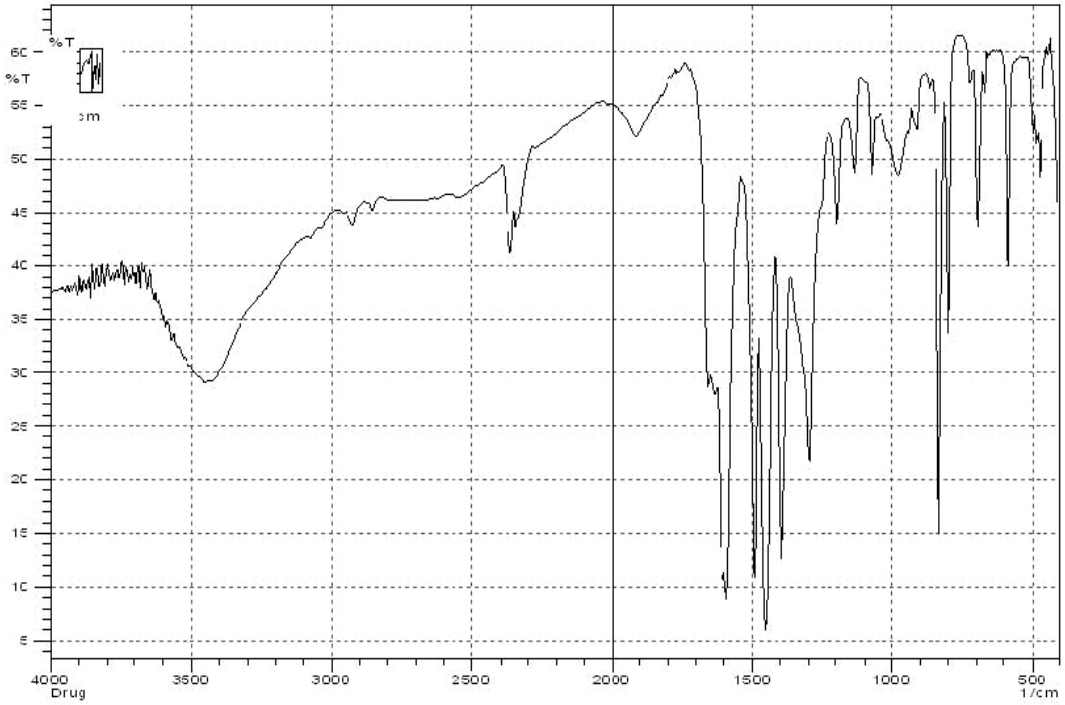


Fig. 2: FTIR Spectrum of olsalazine sodium and combination of excipients

Differential Scanning Calorimetry (DSC) analysis

The DSC thermograms for drug and physical mixture of drug and excipients are represented in figure 3 and 4 respectively. DSC analysis of olsalazine sodium shows the exothermic peak at its melting point i.e. at 249^{0}C. The DSC analysis of physical mixture of drug and excipients revealed negligible change in the melting point of olsalazine sodium in the presence excipients.

This also indicated that there are no changes in its crystallinity of the drug and it may not affect the stability of formulation and it is confirmed that drug is compatible with excipients.

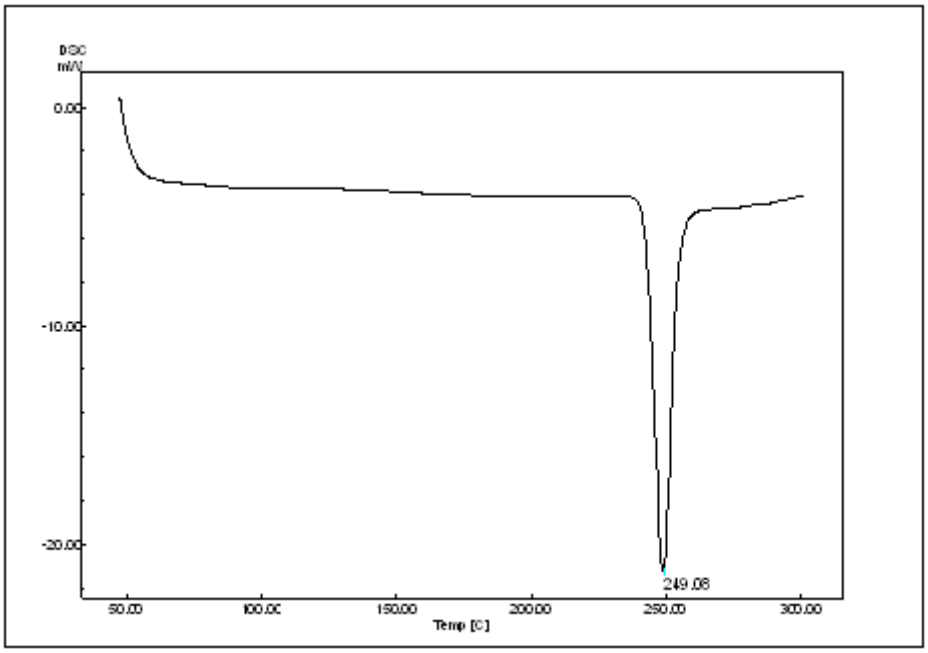


Fig. 3: DSC thermogram of olsalazine sodium

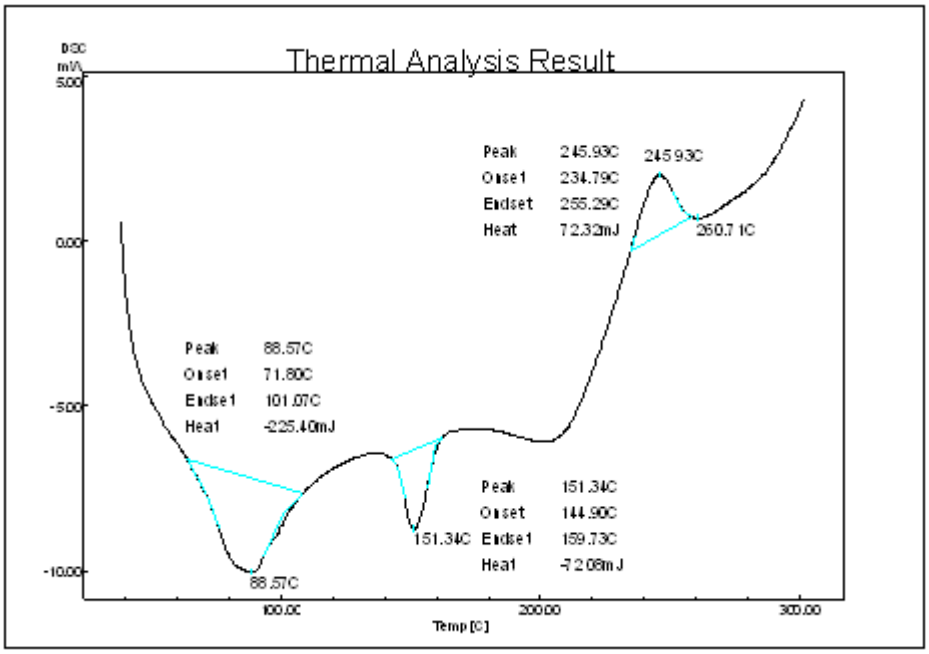


Fig. 4: DSC thermogram of olsalazine sodium combination of excipients

Biodegradation studies of BFM

The degradation of BFM in 4% w/v RCC was more than 2% w/v RCC as the viscosity decrease was proportionately more in 4%w/v RCC. The viscosity measurements revealed that there is more reduction in viscosity with enzyme induction as compared to without enzyme induction, as enzyme induction increased the amount of enzymes produced by bacterial flora. The decrease in viscosity was more with 4% w/v RCC than 2% w/v RCC due to increase in microbial population. The results indicated that 7 days of enzyme induction increase enzyme level markedly as was evident from decrease in viscosity which was due to more complete digestion of BFM. The viscosity decrease was much more than that after 3 days of enzyme induction with 2% w/v RCC and 4% w/v RCC and without enzyme induction. The results of enzyme induction after 7 days indicate excellent bacterial degradation in the colon. Decreased in viscosity of the solution indicates that the mucilage is prone to biodegradation.

Micromeritic properties of olsalazine sodium powder blend

The micromeritic properties of the different batches of olsalazine sodium powder blend indicated that the powder blends were suitable for compression and also free flowing. The angle of repose of all formulations was found in the range of 21.2±0.03 to 24.2±0.04 indicating the granules are freely flowable. Bulk density was found to be 0.41±0.01 to 0.55±0.03 and the tapped density is in range of 0.44±0.11 to 0.67±0.02. Hausner ratio was found to be 1.07±0.02 to

1.18±0.02. Bulkiness was found to be 1.81±0.03 to 2.43±0.01. Carr's Compressibility index of granular bed was found to be less than 25 % indicating good to excellent flow of granules (table 2).

Physiochemical characteristics of colon targeted tablets of olsalazine sodium

The compressed tablets were evaluated for post compressional characteristics like weight variation, thickness, diameter, hardness, drug content, disintegration time and swelling studies. The weight variation study of all the formulation were found to be 398±0.04 to 404±0.02 indicating that, all the weights were within 5% deviation range and passed the weight variation according to IP. Thickness of the tablets were found to be 3.8±0.05 mm to 4.2±0.01 mm, and the diameter of tablets were found to be in the range of 8.05±0.02 mm to 8.27±0.04 mm, batches of different formulation are uniform and reproducible.

The hardness of the tablets was found to be 5.0±0.16 to 5.6±0.16 Kg/cm^2 indicating tablets possess sufficient strength. Further disintegration test was conducted to understand the actual behaviour of the tablets *in vitro*. It was found that, the olsalazine sodium colon targeted tablet did not disintegrate for 2 h in 0.1N HCl but gradual swelling of the tablet was observed. There was no loss of integrity. The friability of formulations was found to be minimum (0.21 % to 0.33 %). Tablet can withstand stress during transport. The drug content of tablet formulations was found to be 99.45±0.05 to 101.91±0.01% which indicates that there is uniform distribution of drug throughout the batch. The results of physicochemical characteristics of olsalazine sodium tablets presented in table 3.

Table 2: Micromeritic characteristics of olsalazine sodium powder blend

Formulation code	Angle of repose(º)*	Bulk density (gm/cm^3)*	Tapped density (gm/cm^3)*	Carr's index (%)*	Hausner ratio (H_R)*	Bulkiness (cc/g)*
F1	22.0±0.04	0.55±0.03	0.67±0.02	17.9±0.03	1.16±0.02	1.81±0.03
F2	24.2±0.04	0.50±0.04	0.58±0.0	13.3±0.58	1.11±0.04	2.01±0.04
F3	23.5±0.06	0.45±0.01	0.50±0.18	9.9±0.02	1.07±0.02	2.22±0.02
F4	23.0±0.02	0.41±0.01	0.44±0.11	10.33±0.58	1.18±0.02	2.43±0.01
F5	21.2±0.03	0.50±0.02	0.59±0.01	15.33±0.47	1.11±0.03	2.02±0.02

*All values are expressed as mean ± SD, n=3.

Table 3: Results of physico-chemical properties of olsalazine colon targeted tablets

Formulation code	Thickness (mm)*	Diameter (mm)*	Hardness (kg/cm^2)*	Friability (%)**	Drug content (%)***	Weight variation (mg)***	DT* (min)
F1	4.0±0.01	8.09±0.01	5.2±0.16	0.23±0.04	99.45±0.05	398±0.07	ND*
F2	3.8±0.05	8.05±0.02	5.2±0.14	0.33±0.03	100.12±0.04	398±0.04	ND
F3	4.2±0.01	8.12±0.03	5.0±0.16	0.21±0.05	101.34±0.05	401±0.05	ND
F4	4.1±0.02	8.27±0.04	5.1±0.14	0.24±0.05	100.43±0.06	399±0.01	ND
F5	4.2±0.01	8.10±0.03	5.6±0.16	0.32±0.01	101.91±0.01	404±0.02	ND

*All values are expressed as mean ± SE, n=5; **All values are expressed as mean ± SE, n=10; ***All values are expressed as mean ± SE, n=20; ND*: not disintegrated till 2 hrs; DT: Disintegration time.

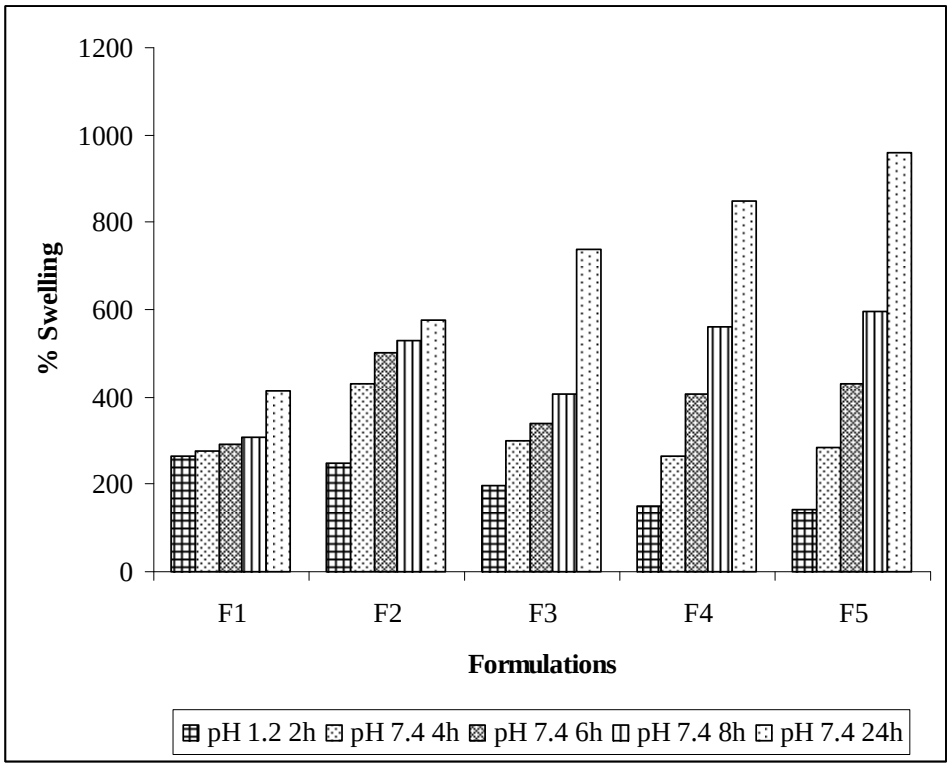


Fig. 5: Swelling behaviour of different batches of olsalazine colon targeted

Swelling behaviour of olsalazine colon targeted tablets

The swelling behaviour indicates the rate at which the formulation absorbs water from dissolution medium and swells. In pH 1.2 the rate of swelling at 2 h ranges from 262.53%–141.47%. However, in 7.4 pH media the % increase in swelling ranges from 416.04%–959.45% at 24h. The results indicated that, as the content of the mucilage increases, the hydration of mucilage increases due to more amount of water diffusion in the mucilage, as a result the % of swelling index increased. As the amount of mucilage increases from (20–100 mg), swelling decreases from 262.53%–141.47% in pH 1.2 media while it increases from 416.04%– 959.45% in pH 7.4. This drastic difference in swelling could be attributed to presence of some hydrophilic ionisable groups like COOH. The experiment clearly shows the swelling nature of the tablets. The tablets appeared swollen almost from the beginning, a viscous gel mass created when they came into contact with the liquid. This indicates the ability of the BFM tablets to hydrate and swell which is an important prerequisite for a colon specific carrier. The resultant swelling evades a diffusion barrier at the surface of the solid dosage form during its passage through the GIT. These hydrated layers of mucilage allow the penetration of colonic enzymes and bacteria which leads to the degradation of the polysaccharide barrier. Thus the swelling of the tablets will help to release the drug successfully in the suitable environment of the colon(figure 5).

***In vitro* drug release study**

In vitro release studies carried out revealed that the formulations containing 5, 10 and 15% w/w of BFM respectively released above 90% of drug within 5 h indicating drug released in stomach and small intestine. The release pattern also showed that drug release was lesser in formulation F4 and F5 containing higher proportion of BFM (20and 25%w/w). Drug release was comparatively higher in SIF ranging from 22.51% and16.34% for F4 and F5 respectively. This result clearly indicates that higher the proportion of BFM higher is the ability to prevent the drug release in upper GIT. Release of drug increased significantly in SCF from 56.18% to 61.2%, release rate at this environment was more for formulation F4 and F5. These release patterns clearly indicates the potential of the BFM to be a suitable colonic drug delivery carrier. In absence of RCC, results of the drug release studies in 0.1N HCl for 2 h and pH 7.4 Sorensen's phosphate buffer indicate that BFM is capable of protecting the drug at higher concentrations (20% and 25% w/w). The formulation F5 containing 25% w/w of BFM released 56.18 % of drug in 24 h, it was observed that, tablet swelled after 10 min and remained intact throughout the 24 h dissolution study. The results has given an insight that BFM at 25%w/w (F5) could be used for development of colon matrix tablets of olsalazine sodium with gradual increments in their amount to optimize the formulation of colon tablets(figure 6).

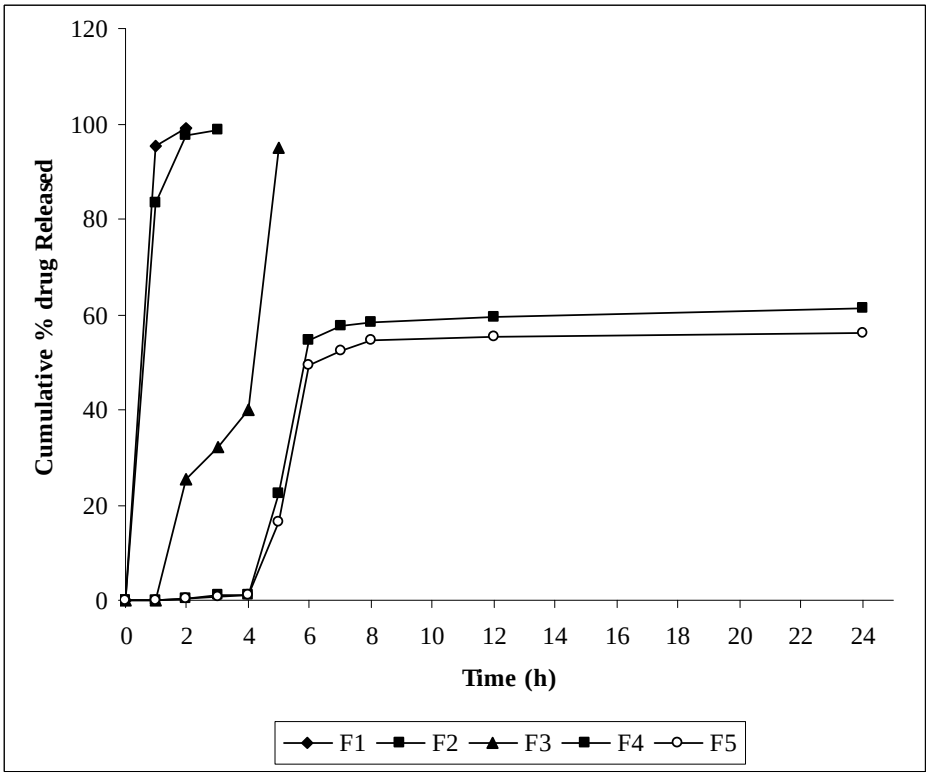


Fig. 6: Comparison of i*n vitro* release profile of different batches of olsalazine sodium colon targeted tablet without RCC

***In vitro* drug release study with 4% (w/v) rat caecal matter**

In order to study the susceptibility of matrix tablet to colonic enzymes, the medium were replaced with phosphate buffer saline of pH 6.8 containing 4% rat caecal content and studied for 19 h. With this step it would be fair to understand whether the drug release is influenced by dissolution medium or the rat caecal contents medium. Considering the drug release in pH 6.8 Sorensen's phosphate buffer and protection provided by the carrier in simulated upper GI fluids, formulation F5 was selected for further studies in SCF containing 4% (w/v) rat caecal matter as it predominantly renders anaerobic environment. The release pattern was similar during the first 5 h in SGF and SIF, The presence of rat caecal contents improved the drug release when compared with control i.e pH 6.8 Sorensen's phosphate buffer without rat caecal contents. The percent drug released from matrix tablets after 24h in pH 6.8 Sorensen's phosphate buffer was increased to 56.18% without RCC and 79.81% with 4% w/v RCC. This indicates that polysaccharidases are present in caecal matter that metabolizes BFM.

This clearly reveals the susceptibility of the BFM for biodegradation in anaerobic environment of colon which is present *in vivo.* The colonic bacteria are predominately anaerobic in nature and secrete enzymes that are capable of metabolizing substrates such as carbohydrates and proteins that escape the digestion in the upper GI tract. Most common mechanisms of microbial activation in the colon are azo-reduction and glycosidic-bond hydrolysis. BFM consists of the sugars galacturonic acid, galactose, glucose and glucuronic acid. The results showed that drug release is less in upper gastrointestinal

tract and comparatively more in colonic environment containing 4% (w/v) rat caecal matter can be clearly related to the above mentioned facts. At the end of 24 h the matrix tablet integrity did not retained. The polymer matrix was susceptible to anaerobic enzymes; the viscous gel of the matrix weakens to release the drug. From this data it can be concluded that BFM can be used for targeting the drug to the colon rather than sustaining or controlled release of drug (figure 7).

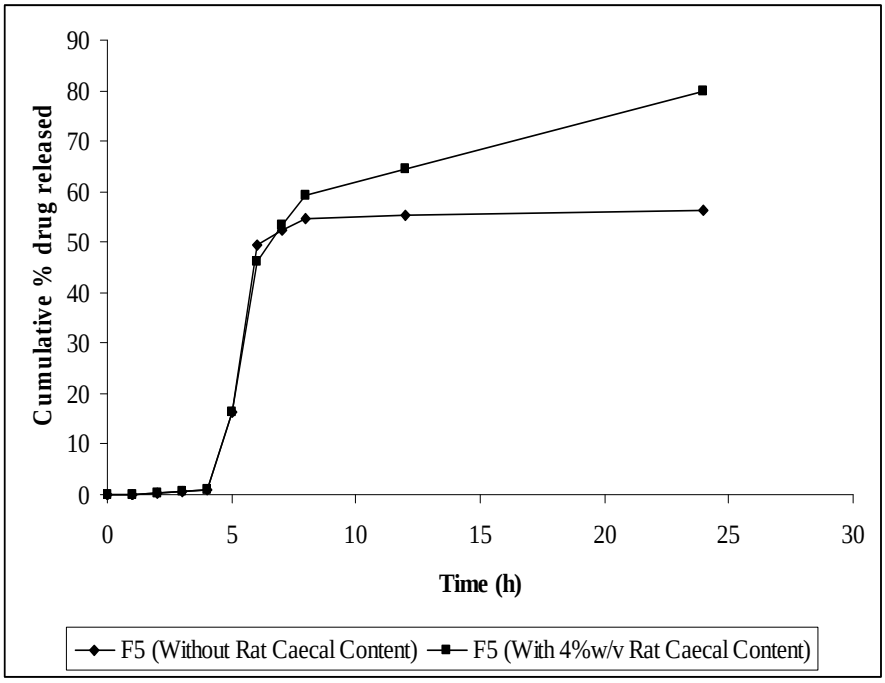


Fig. 7: Comparison of i*n vitro* release of optimized batch of colon targeted tablet with and without 4%w/v RCC

Drug release kinetics

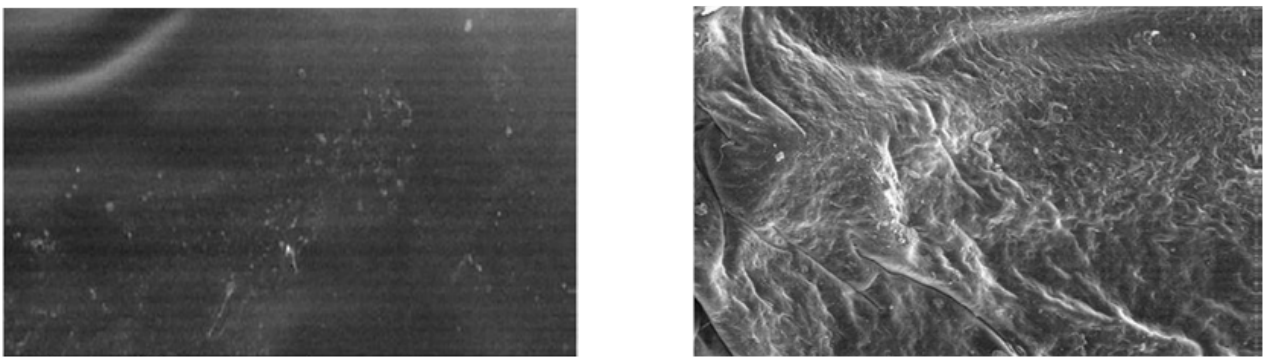
In vitro release kinetics of the formulation F5 was subjected to mathematical treatment for zero order, 1st order, and Higuchi and Peppas model. Based upon the evaluated r^2 value, release pattern of the optimized formulation (F5) was best fitted to Korsmeyer-Peppas model. For formulation F5 (n = 1.310) in case of Peppas model indicating the drug transport mechanism as Super Case II transport. This model describes the release of those polymeric dosage forms when more than one type of release phenomenon could be involved. Therefore in the present case the release of drugs from the tablets is by swelling and erosion which is also in accordance to the swelling index and *in vitro* drug release data.

Scanning Electron Microscopy studies (SEM)

In order to elucidate the mechanism of drug release from optimized formulation F5, surface of optimized tablet, both before and after dissolution studies, was studied using scanning electron microscope (SEM). Results of SEM studies of Formulation F5 before and after *in-vitro* dissolution studies were shown in figure 8. From this it is evident that the formation of pores in the membrane after coming in contact with simulated colonic fluid and these pores will act as orifice for the release of drug in colon.

***In vivo* targeting efficiency**

To strengthen the *in vitro* release study finding, *in vivo* efficiency study was carried out. It can be concluded from the X-ray images that the tablet (F5) was intact and slight swelling was observed in the first 2 h. This observation is similar to *in vitro* drug release as drug release was low during this period. After 5 h the tablet still appeared to be intact with further swelling showing the increasing tendency of swelling in higher pH as par the *in vitro* swelling study. At 7 h as the tablet approaches colon, disintegration was observed indicating the enzymatic degradation of the BFM and finally at 24 h the tablet disappeared which is also in accordance to the *in vitro* data. Thus *in vivo* study confirms the finding of *in vitro* study. The X-ray image of olsalazine sodium colon targeted tablet in different parts of GIT is shown in figure 9.



Before *in vitro* dissolution study **after *in vitro* dissolution study**

Fig. 8: SEM photograph of olsalazine sodium colon targeted matrix tablet (F5)

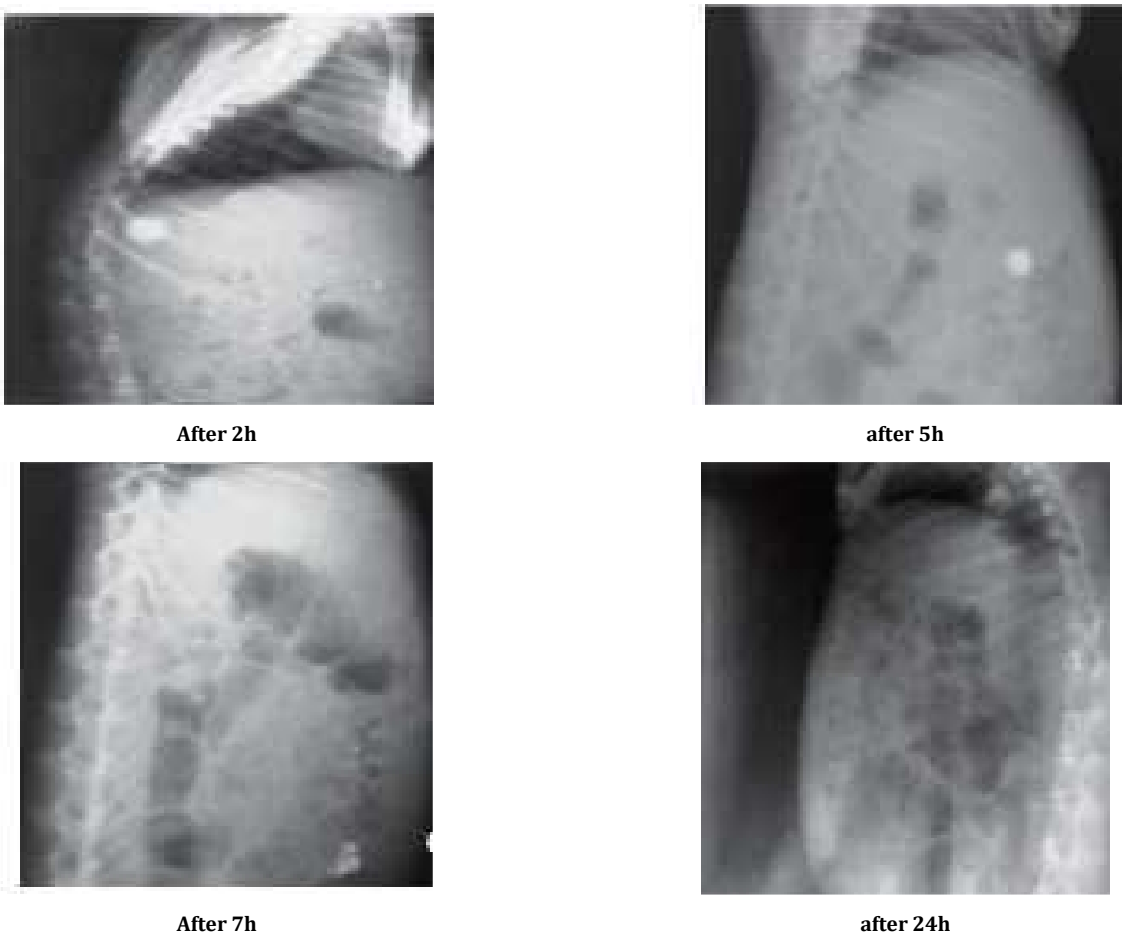


Fig. 9: X-ray image of olsalazine colon tablet (F5) in different parts of GIT in rabbit

Stability study

In view of the potential utility of optimized F5 formulation for targeting Olsalazine sodium to colon, stability studies were carried out at 40°C/75% RH for 3 months (climatic zone IV conditions for accelerated testing) to assess their long term stability. Accelerated stability studies were carried out according to ICH guidelines. At the end of the testing period, the tablets were observed for changes in physical appearance, analyzed for drug content and subjected to in vitro drug release studies. No visible changes in the appearance of the tablets were observed at the end of the storage period. Results also indicated that the tablets did not show any significant changes in hardness, weight variation and friability. The drug content was found to be 98.4±0.01%.

At the end of 24 h of dissolution testing, the percentage release of olsalazine sodium from F5 formulation before storage was 79.82% whereas that released from the F5 formulation after storage was 78.21±0.80%. There was no significant difference in the % cumulative release of the drug from F5 tablet formulation after storing for 3 months at 40°C/75% RH indicating that the formulation could provide a minimum shelf-life of 2 years (figure 10).

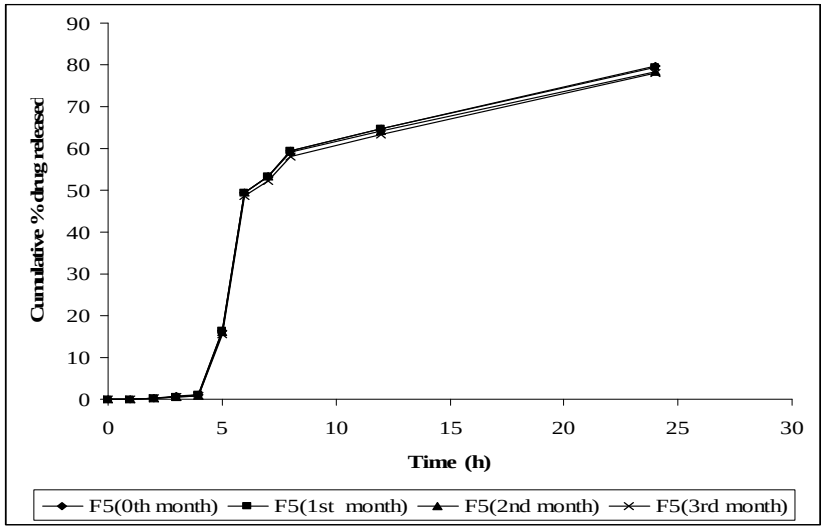


Fig. 10: Comparison of *in vitro* release profile of F5 during different durations of stability study

CONCLUSION

During the last decade there has been interest in developing site-specific formulations for targeting drug to the colon. The colon has gained attention on the delivery of drugs not only for the treatment of local diseases associated with the colon but also for its potential for the delivery of proteins and therapeutic peptides sensitive to the enzymes in both the stomach and small intestine. Among the different approaches to achieve colon specific drug delivery, the use of polymers, specifically biodegraded by colonic bacterial enzymes holds promise. The *in vitro* and *in vivo* study indicated that the formulation F5 containing 25%w/w of BFM was limited the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment. *In-vitro* biodegradability studies suggested that BFM is degraded in the presence of rat caecal contents under conditions mimicking colon. *In-vitro* drug release studies under conditions mimicking mouth to colon transit, demonstrated the ability of BFM to release the drug in pH 6.88 Sorenson's phosphate buffer with RCC. Thus on the basis of the above mentioned findings it could be concluded that BFM could be successfully used in colon specific delivery systems.

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