Modified Katira Gum for Colon Targeted Drug Delivery

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ABSTRACT: The aim of the this study is to develop colon targeted drug delivery systems for Ibuprofen using grafted katira gum as a carrier. Polyacrylamide-grafted katira gum was synthesized by grafting acrylamide onto katira gum in presence of varying concentration of ceric ammonium nitrate (CAN) as initiator. Elemental analysis, FTIR, TGA, DTA, DSC, and SEM were used to characterize the grafting of acrylamide onto katira gum. Matrix tablets containing various proportions of grafted katira gum were prepared by wet granulation method. All the formulations were evaluated for hardness, drug content, swelling index, and *in vitro* release studies in simulated gastric fluid, small intestinal fluid, and simulated colonic fluid with and without enzymes. Ibuprofen was used for controlled release study. The drug release mechanism was studied by fitting into Peppas model and was found to be supercase-II transport. Matrix tablets containing 0.3 g of CAN/gm of acrylamide showed optimum value and retained its physical integrity in simulated gastric, small intestinal and colonic fluid, where as other composition disintegrated within 2 h of dissolution testing in pH 1.2 buffer, simulated gastric fluid. The results of this study indicates that Ibuprofen matrix tablet containing 60 wt % composition of the above grafted katira gum would be potential formulations in delivering the drug to the colon and the more susceptible for enzymatic degradation. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2644–2651, 2011

Key words: katira gum; acrylamide; drug delivery systems; FTIR; TGA; DSC

INTRODUCTION

Delivery of drugs to the colon has been extensively investigated during the last decade. A number of diseases, e.g., Crohn's disease, ulcerative colitis, and the irritable bowel syndrome can be treated most efficiently by local delivery of drugs.[1] Site-specific systems might also reduce systemic absorption and side effects.[2] It has also been suggested that colonic delivery of orally administered protein and peptide drugs might be possible, because enzyme activity is low in the colon. Colon targeted drug delivery systems would also be advantageous when a delay in absorption is desirable from a therapeutic point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina, and rheumatoid arthritis.[3–5]

Various approaches have been proposed for targeted colon drug delivery, namely pH- and time-dependent systems, pressure-controlled release systems, osmotic systems, prodrugs, and polysaccharide-based delivery systems.[6–9] The pH approach has been shown to lack site-specificity because of inter/intra subject variation and the similarity of the pH between the small intestine and the colon.[10,11] Timed-release systems depend on the relative consistency of the small intestinal transit times, but the high variability in gastric retention times makes prediction of the accurate location of drug release difficult.[12] Prodrugs and polysaccharide-based delivery systems depend on the enzymatic degradation carried out by the inherent bacterial flora present in the colon, thereby resulting in drug release. The enzyme trigger mechanism in such delivery systems makes them highly site-specific. Prodrugs, however, are considered as new chemical entities from a regulatory perspective, which requires a detailed toxicological study to be performed, before being used as drug carriers.[13]

Natural polysaccharides, however, fall under the category of "GRAS" (Generally regarded as safe), thus resolving the general problems associated with safety. Natural polysaccharides, including chitosan, pectin, guar gum, xanthum gum, dextran, and inulin remain undigested in the stomach and small intestine and are degraded by the huge numbers of anaerobic microflora in the colon[8] e.g., bacteroides, bifidobacteria, eubacteria, clostridia, to smaller monosaccharides, which are then used as energy source by the bacteria. In this study, the use of an inexpensive, naturally occurring, and abundantly available polysaccharide, katira gum, as a release-retarding polymer for colon targeted drug delivery is explored.

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Katira gum is a novel heteropolysaccharide isolated from Cochlospermum religiosum and consist of D-galactose, D-galacturonic acid, and L-rhamnose in a molar ratio 2 : 1 : 3, respectively, and traces of a ketohexose.[14] The modifications of katira gum by grafting it with acrylamide using different amount of initiator (CAN) are reported. For *in vitro* release tests, sequential liquids simulating the physiological variation of pH were used and the effect of the presence or absence of pectinex ultra spl enzyme having galactouronidase activity, in the simulated colonic medium was evaluated, to better understand the influence of these enzymes on the drug release and to investigate the drug release mechanism.

Ibuprofen was chosen as a model drug because it is readily absorbed throughout the gastrointestinal tract. The drug has low water solubility. The drug is also a candidate for an intentional delayed absorption from a dosage form ingested at bedtime for the treatment of arthritis, which may have peak symptoms in the early morning.[15]

MATERIALS AND METHODS

Materials

Ibuprofen [(S) (+) isomer] gratis samples as supplied from Doctor Organic Chemicals, Tanuka, India. Katira gum was obtained from Indian Institute of Resin and Gums, Ranchi, India, and was of pharmacopeia quality (USNF). Acylamide, ceric ammonium nitrate (CAN) was obtained from S.D. Fine Chem., Mumbai, India. Pectinex Ultra SP-L (pectinolytic enzymes, extracted from Aspergillus niger and having an activity of 26,000 PG/ml at pH 3.5) was kindly supplied by Novozymes South India, India. Other materials used in the study, such as microcrystalline cellulose (Avicel, pH-101), starch, magnesium stearate and talc were of pharmacopeia quality (USNF).

Synthesis of polyacrylamide-grafted katira gum

Polyacrylamide-graftedguar gum was synthesized as follows: 3 g of katira gum were taken in 150 mL distilled water and stirred in magnetic stirrer for dissolution. After 24 h the swelled mass was mixed with 0.9 mL of conc. HNO_3 and heated in water bath at 60°C to complete the dissolution of swelled gum. Then 15 g of acrylamide was dissolved in 100 mL of water and added to the above solution in stirring condition. After half an hour CAN (initiator) of required quantity as shown in Table I was dissolved in 100 mL of water and added to above solution. The reaction mixture was heated to 60°C and the heating and stirring was continued up to 5 h. Acetone was added as a nonsolvent to precipitate the polymer, which was washed with 30% aqueous methanol to remove the homopolymer of acrylamide. The grafted gum was purified twice by dissolving in small quantity of water and reprecipitated by acetone. The solid polymer obtained was dried at 40°C for 24 h in vacuum oven and stored in a desiccator for further use. These graft copolymers prepared from different ratios of katira gum to initiator (CAN) were named respectively, as G1, G2, and G3. The percentage of grafting (%G) was calculated according to the following formula.[16,17]

TABLE I
Synthetic Details of Polyacrylamide-Grafted-Katira Gum

Sample identification	Mass of katira gum (g)	Mass of acrylamide (g)	Mass of initiator (ceric ammonium nitrate) (g)
G1	3	15	0.15
G2	3	15	0.45
G3	3	15	0.60

$$\% \text{ Grafting} = \frac{W_{AM} - W_G}{W_G} \times 100, \qquad (1)$$

$W_{AM} =$ Mass of graft copolymer and $W_G =$ Mass of original gum.

Characterization of gels

Elemental analysis

The elemental analysis of the vacuum dried grafted and ungrafted katira gum were carried out with an elemental analyzer (Elementar, Germany model: Vario EL111).

Fourier transform infrared spectroscopy

The FTIR spectra of pure katira gum and grafted katira gum (G1,G2,G3) were recorded in solid state using KBr pellets with a Shimadzu, Model IR-Prestige-21, Japan. FTIR spectra were scanned in the wavelength range 400–4000 cm^{-1}.

Differential scanning calorimetry

Differential Scanning Calorimeter (TA Instruments, model: DSCQ10) was used to study the thermal characteristics of the grafted and ungrafted katira gum. Approximately 4–5 mg of gum samples were weighed into aluminum pans and sealed. Empty closed aluminum pan was used as the reference cell. The thermal behavior was recorded from –60°C to +170°C at a heating rate of 10°C/min. All the samples were equilibrated for 15 min at the starting temperature. The thermal analysis was carried out in nitrogen atmosphere.

TABLE II
Composition of Ibuprofen Matrix Tablets Containing Grafted Katira Gum

Ingredients	Quantity (mg) present per each matrix table								
	G1–10	G1–20	G1–30	G2–10	G2–20	G2–30	G3–10	G3–20	G3–30
Ibuprofen	100	100	100	100	100	100	100	100	100
G1	20	40	60	–	–	–	–	–	–
G2	–	–	–	20	40	60	–	–	–
G3	–	–	–	–	–	–	20	40	60
MCC	54	34	14	54	34	14	54	34	14
Starch	20	20	20	20	20	20	20	20	20
Talc	4	4	4	4	4	4	4	4	4
Mg stearate	2	2	2	2	2	2	2	2	2
Total	200	200	200	200	200	200	200	200	200

Thermal analysis (TGA and DTA)

TGA and DTA analyses of pure katira gum (KG) and grafted katira gum(G1,G2,G3) were done using Shimadzu, Model DTG-60, Japan from room temperature to 800°C at the heating rate of 10°C/min under a constant flow of nitrogen.

Preparation of ibuprofen matrix tablets

Matrix tablets of Ibuprofen were prepared by wet granulation method.[18] Microcrystalline cellulose (MCC) was used as diluent and a mixture of talc–magnesium stearate (2 : 1) was used as lubricant and katira gum was included in the formulations in various proportions. The compositions of different formulations used in the study containing 100 mg of Ibuprofen in each case are shown in Table II.

In all the formulations, Grafted Katira gum was sieved separately and mixed with Ibuprofen and MCC. The powders were blended and granulated with 10% starch paste. The wet mass was passed through a mesh (1680 μm) and the granules were dried at 50°C for 2 h. The dried granules were passed through a mesh (1190 μm) and these granules were lubricated with a mixture of talc–magnesium stearate (2 : 1). The lubricated granules were compressed using 9 mm round, flat, and plain punches on a single station tabletting machine (M/s Cadmach Machinery, India). Matrix tablets of each composition were compressed and tested for their hardness, drug content, and drug release characteristics with a suitable number of tablets for each test. The hardness of the matrix tablets was determined by using the Monsanto Hardness Tester.

Degree of swelling of tablets

The tablets in a wire basket were put into a 250 mL beaker containing 200 mL of pH 7.4 phosphate buffer and 6.8 phosphate buffer and were allowed to swell at 37°C.[19] Tablets were periodically removed and changes in weight were measured. The swelling ratio was then calculated using the following formula.

$$\text{S.I.} = \frac{M_t - M_o}{M_o} \times 100, \quad (2)$$

Where, S.I = Swelling Index or swelling ratio, M_t = weight of tablet at time t, M_0 = weight of tablet at time $t = 0$, respectively.

In vitro drug release studies

In vitro release studies were carried out using the USP paddle apparatus at 37 ± 0.5°C and at a stirring rate of 50 rpm in 700 mL of dissolution medium according to the following condition, to mimic the mouth-to-colon transit: 2 h in pH 1.2, simulating the gastric juice and next 2 h in pH 6.8 phosphate buffer, mimicking the small intestine fluid and next 4 h in pH 7.4 phosphate buffer, simulating the colonic environment in the absence or presence of 3.5 mL pectinex ultra spl having galactouronidase activity, added to evaluate *in vitro* the effect of galactouronic acid in katira gum, breakdown which *in vivo* is brought about by colonic bacteria.[20] At predetermined time intervals, samples were withdrawn and spectrometrically assayed for drug concentration at 221 nm.

Scanning electron microscope

SEM images of katira gum and grafted katira gum (G1,G2,G3) were obtained using a JEOL, Model JSM6390LV, Japan. The acceleration voltage was 4–5 kV. Magnifications were in the range of 500 to 10,000X.

RESULTS AND DISCUSSION

Synthesis of polyacrylamide-grafted katira gum

Graft copolymers based on katira gum were synthesized by grafting acrylamide onto polysaccharide molecules by a radical polymerization using CAN as the initiator. A series of graft copolymers were

TABLE III
Elemental Analysis Result of Grafted and Ungrafted Katira Gum

Sample identification	%C	%N	N/C ratio
KG	36.6	0.081	0.002
G1	41.48	14.47	0.348
G2	41.51	14.83	0.357
G3	39.81	14.89	0.374

synthesized by varying the initiator concentration and maintaining constant concentration of acrylamide and polysaccharide. With the increase in the initiator concentration the yield of grafted polymer also increased continuously, as expected. The percentage of grafting for G1, G2, G3 was calculated by eq. (1) and were found to be 245, 332, and 430%, respectively.

Elemental analysis (CHN)

The acrylamide monomer contains nitrogen in each monomer unit. Hence the grafting sample should have higher nitrogen content than ungrafted sample. Table III shows the percentage nitrogen and the ratio of N/C of the grafted and ungrafted sample. This shows that the grafting sample has higher nitrogen content and the percentage of nitrogen increases with increase in catalyst concentration.

FTIR spectrum

The FTIR spectra of ungrafted and grafted katira gum are shown in Figure 1. All the graphs are showing broad peak in the range of 3000–3500 $cm^{-1.}$ This is due to hydrogen bond stretching vibrations (O—H or N—H bonds) of ungrafted and grafted sample. In case of katira gum the strong peak near 1750 cm^{-1} is due to the carbonyl group stretching frequency, which shifts below 1700 cm^{-1} is due to the extended conjugation with lone pair of nitrogen in amide group. This supports the grafting of acrylamide on katira gum sample.

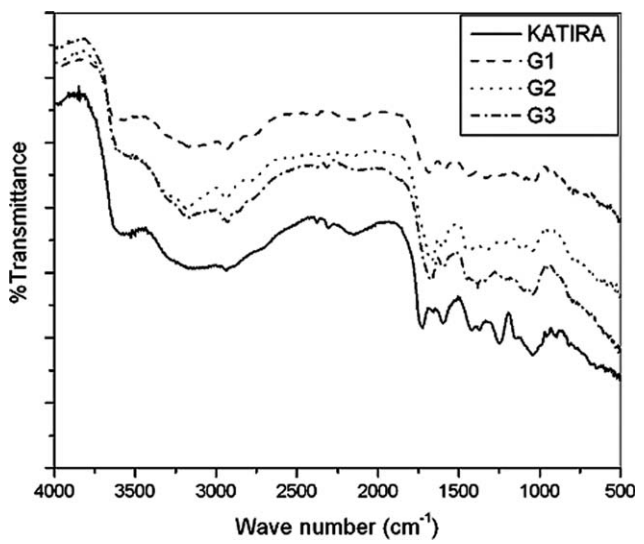

Figure 1 FTIR spectra of ungrafted and grafted katira gum.

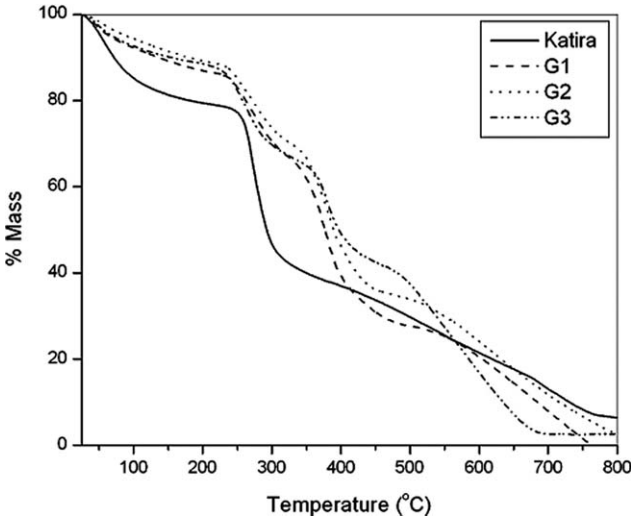

Figure 2 TGA thermogram of ungrafted and grafted katira gum sample.

Thermal analysis (TGA and DTA)

TGA thermograms of ungrafted katira gum and grafted katira gum are presented in Figure 2. In case of katira gum three major decomposition steps are observed. One is from room temperature to 150°C. This is mainly due to the adsorbed moisture present in the sample. The second step starts near 250°C and is continued up to 320°C. Third step is above 320 to 780°C, which is comparatively slow. But in case of grafted katira gum (G1, G2, G3) four decomposition steps are observed. One from room temperature to 250°C, which is due to the adsorbed moisture and the second, is from 250 to 350°C. The third is from 350 to 430°C. Fourth step is comparatively slow and is up to 550°C and the last step is above 550°C. The residue for grafted sample is lower compare to ungrafted sample. The degradation behaviors of grafted samples are different with ungrafted sample. This is due to the structural differences of grafted sample than ungrafted sample.

DTA thermograms of ungrafted katira gum and grafted samples are presented in Figure 3. In all the cases the first endothermic peak near 65°C is due to the adsorbed moisture present in the sample. The interesting point is near 250°C an exothermic peak observed for ungrafted katira gum where as it is endothermic for grafted sample. This is due to the presence of amide group of acrylamide near by the hydroxyl group of katira gum, which changes the

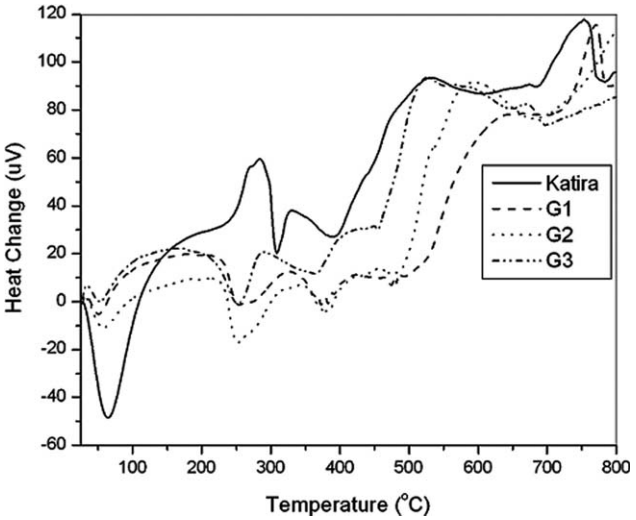

Figure 3 DTA thermogram of ungrafted and grafted katira gum sample.

degradation of gum sample. The change in TGA and DTA curve for grafted sample compare to katira gum supports the grafting has taken place on katira gum.

Differential scanning calorimetry

Figure 4 is the DSC curve of ungrafted and grafted katira sample. All the graph shows the endothermic peak after 50°C and is continued above 100°C. The endothermic peak for katira gum is at 125°C whereas for grafted sample it is near 100°C. This endothermic peak is due to the presence of adsorbed moisture in the sample.

The grafted sample shows lower amount of endothermic peak than ungrafted sample. A new peak near 75°C is observed for grafted sample (KG3) is due to the glass transition temperature of polyacrylamide. This indicates the grafting has taking place on the katira gum sample.

Scanning electron microscopy

A comparative study of the scanning electron micrographs of ungrafted katira gum and grafted katira gum are shown in Figure 4. Although the samples were prepared in the same environment, but the surface structures are different and this affects the rate of dissolution and swelling of the solid sample.

Swellability studies

Swelling study was performed on formulations containing G2–10, G2–20, and G2–30 in pH 6.8 and pH 7.4 buffer and these results are presented in Figures 5 and 6, respectively. Matrix tablets containing G1(10, 20, and 30%) and G3 (10, 20, and 30%) were also subjected to swellability studies but it gets disintegrated after 1 h of swelling study. G2–10 and G2–20 exhibit a lesser swelling ratio when compared to G2–30 in both pH 6.8 and pH 7.4 buffer. The result shows that in case of G2–30, the swelling increases with increase in time in both buffers. But in case of G2–10 and G2–20, the swelling index was initially increased and gradually decreases in both pH 6.8 and pH 7.4 buffer.

In vitro drug release studies

The matrix tablets were prepared by applying a force of 350 psi of compression and the hardness of the tablets was found to be in the range of 4.5–5.0 to kg/cm^2. To understand the release profiles of the drug from the tablets, dissolution experiments were performed according to the condition described in materials and methods. The plot of cumulative drug release vs. time for G2 (10%, 20%and 30%) are shown in Figure 7.

Matrix tablets containing G1(10%, 20% and 30%) and G3 (10%, 20% and 30%) were found disintegrated within 1 h of dissolution testing in pH 1.2 buffer, whereas the matrix tablets containing G2 (10, 20, and 30%) retained their shape for up to 8 h of dissolution testing. The cumulative percentage drug release after 8 h was 90.92% ± 1.73 for G2–10 and 55.34% ± 0.17.% for G2–30. In case of G2–20, almost all the drug was released at 5th hour of dissolution study in pH 7.4, simulated colonic fluid as shown in Figure 8.

With the aim of evaluating the influence of the katira gum biodegradation process on the drug release profile, we performed experiments by adding in the simulated colonic medium a commercially available mixture of specific enzymes (Pectinex Ultra

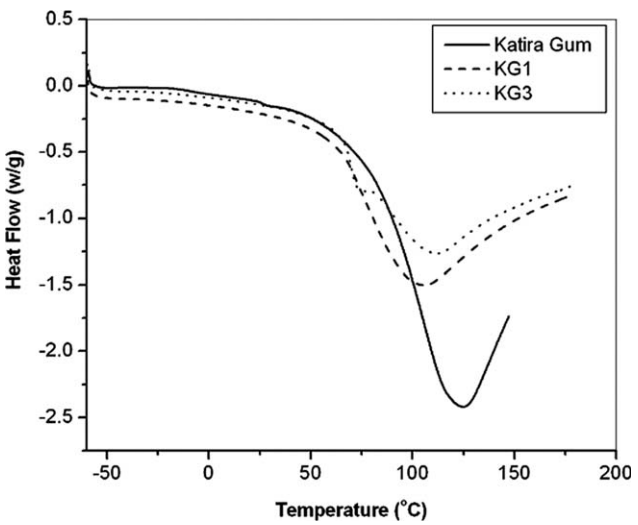

Figure 4 DSC thermogram of ungrafted and grafted katira gum sample.

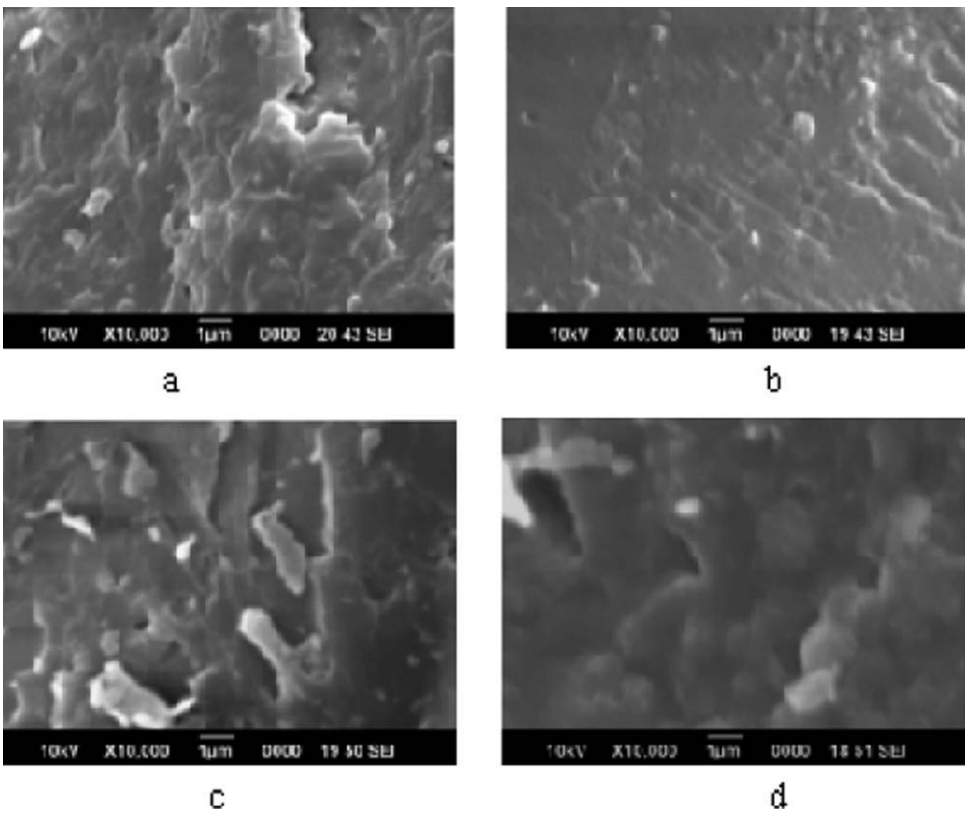

Figure 5 SEM micrographs of tablets containing (a) Katira gum, (b) G2–10, (c) G2–20, (d) G2–30 at 10,000X magnification.

SP-L), whose pectinolytic (galactouronidase) activity showed to be closely correlated with that of the Bacteroides ovatus, the main colon producer of pectinolytic enzymes.[21] The drug release curves obtained from experiments in the presence of enzymes (Fig. 9) were clearly different from those previously obtained from the same tablets in the absence of enzymes, thus confirming the actual potential of katira gum in specific colon delivery.

Release kinetics

To investigate the mode of drug release from, the release data were analyzed with the following mathematical models: zero-order kinetic [eq. (3)], square root of time equation (Higuchi equation, eq. (4)), and Korsmeyer equation [eq. (5)].

$$Q = k_o t \tag{3}$$

$$Q_t = k_H t^{1/2} \tag{4}$$

$$M_t/M_\infty = kt^n \tag{5}$$

In the equations, Q is the percent of drug released at time t and k_0, k_H are the coefficients of the equations, where M_t/M_∞ is the fraction of drug released at time t, K is a kinetic rate constant, and n is the diffusional exponent that characterizes the mechanism of drug release.

For cylindrical systems, if $n = 0.45$ it suggests the Fickian diffusion; if $0.46 < n < 0.89$, it suggests the anomalous (non-Fickian) transport, for $n = 0.89$, the zero-order release is possible and if $n > 0.89$, a supercase- II transport is operative. To predict and correlate the release behavior of drugs from the hydrophilic matrix of this study, it is necessary to fit them into release kinetic profiles. (Fickian,

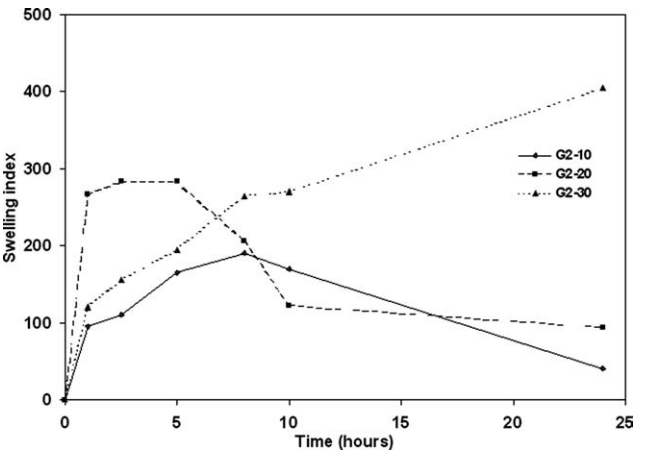

Figure 6 Mean (±S.D.), Swelling index of matrix tablets containing grafted katira gum in 6.8 buffer.

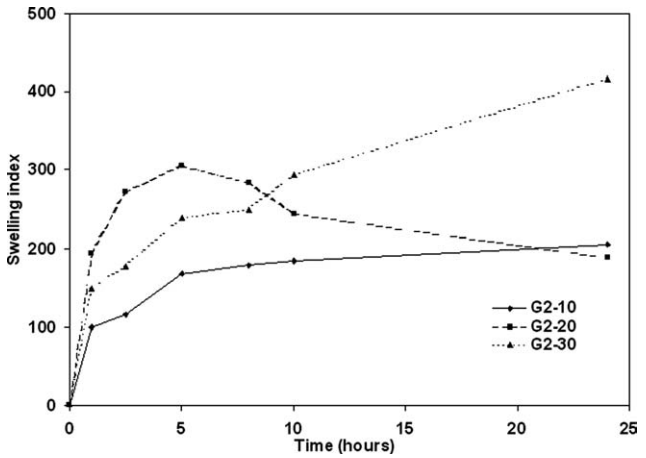

Figure 7 Mean (±S.D.), Swelling index of matrix tablets containing grafted katira gum in 7.4 buffer.

anomalous or super-Case-II).[22] This will facilitate the understanding of mode of drug release, such as whether the release is because of only diffusion or only erosion or caused by both diffusion and erosion. The *n* values calculated for selected formulations (G2–10, G2–20, G2–30), the *n* values are found to be 1.5, 1.7, and 1.4 and is shown in Table IV, indicating a supercase- II transport (corresponds to erosion and relaxation of swollen polymer layer).

DISCUSSION

Matrix tablets containing G1 (10, 20, and 30%) and G3 (10, 20, and 30%) were found disintegrated within 2 h of dissolution testing in pH 1.2 buffer, simulated gastric fluid, whereas matrix tablets containing G2 (10, 20, and 30%) retain their physical integrity in simulated gastric, small intestinal, and colonic fluid. The results of the study indicate that Ibuprofen matrix tablet containing G2–30 katira gum would be potential formulations in delivering the drug to the colon.

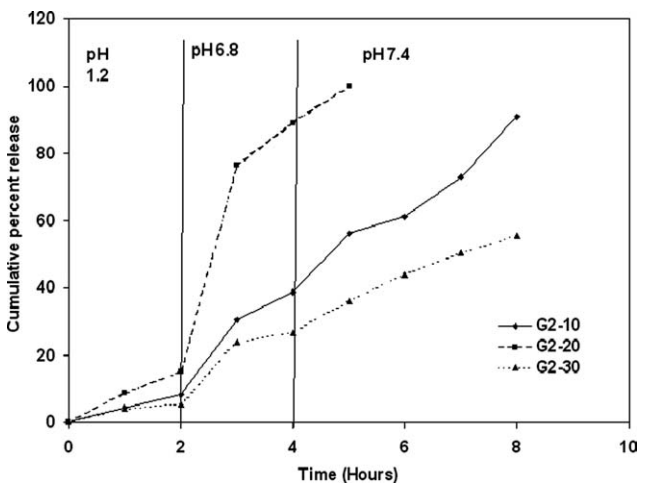

Figure 8 Mean (±S.D.) percent of Ibuprofen released from matrix tablets (n=3) of grafted katira gum in dissolution study without enzymes.

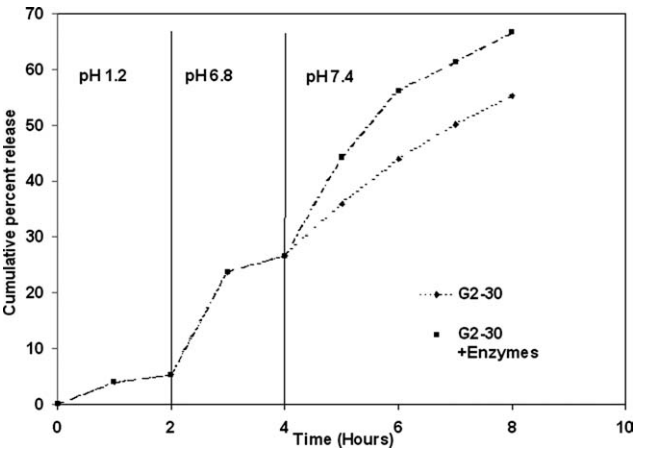

Figure 9 Mean (±S.D.) percent of Ibuprofen released from matrix tablets (*n*=3) of grafted katira gum (G2–30) in dissolution study with and without enzymes.

Finally, the results stressed the importance of using appropriate dissolution test conditions to adequately characterize the release behavior of drug delivery systems endowed with a microflora-activated drug release triggering mechanism. To more fully characterize these delivery systems, further experiments is under progress to investigate the effect of *in vitro* testing conditions in depth, including the presence and, the pectinolytic enzymes concentration, the presence and concentration of animal caecal content.

CONCLUSION

This investigation was performed to develop the colon targeted drug delivery systems for Ibuprofen using a novel polysaccharide, katira gum. The grafting of katira gum was achieved by grafting katira gum with acrylamide using different initiator concentration, i.e., CAN. Grafting was ascertained by elemental analysis, FTIR, TGA, DTA, DSC, and SEM. Swelling study indicated that G2–30 has a maximum swelling index when compared to G2–10 and G2–20

TABLE IV
Kinetic Parameters of Drug Release from G2-10, G2-20, G2-30

Formulation code	Zero order		Highuchi		peppas		
	k_0	r^2	k_0	r^2	n	k	r^2
G2–10	11.68	0.979	33.17	0.849	1.525	4.168	0.964
G2–20	22.9	0.907	48.59	0.767	1.707	7.550	0.905
G3–30	7.49	0.976	21.57	11.84	1.401	3.451	0.930

in both pH 6.8 and pH 7.4 buffers. The result implies that as the concentration of grafted gum in formulation increases in the formulations, increase in swelling index was observed. Among the examined grafted katira gum, G2–30 was the most interesting candidate for specific colonic delivery and the more susceptible to enzymatic degradation. The rapid enzymatic breakdown of G2–30 led to a rapid erosion of the tablets and to a consequent fast release of the drug.

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References

1. Kinget, R.; Kalala, W.; Vervoort, L.; Van Den Mooter, G. J Drug Target 1998, 6, 129.
2. Rubinstein, A. Crit Rev Therapy Drug Carr Syst 1995, 12, 101.
3. Rubinstein, A.; Nakar, D.; Sintov, A. Int J Pharm 1992, 84, 141.
4. Halsas, M.; Hietala, J.; Veski, P.; Jurjenson, H.; Marvola, M. Int J Pharm 1999, 189, 179.
5. Halsas, M.; Penttinen, T.; Veski, P.; Jurjenson, H.; Marvola, M. Pharmazie 2001, 56, 718.
6. Xi, M. M.; Zhang, S. Q.; Wang, X. Y.; Fang, K.; Gu, Y. Int J Pharm 2005, 298, 91.
7. Chourasia, M. K.; Jain, S. K. J Pharm Sci 2003, 6, 33.
8. Chourasia, M. K.; Jain, S. K. Drug Deliv 2004, 11, 129.
9. Sinha, V. R.; Kumaria, R. Eur J Pharm Sci 2003, 18, 3.
10. Ashford, M.; Fell, J.; Attwood, D.; Sharma, H.; Woodhead, P. Int J Pharm 1993, 95, 193.
11. Leopold, C. S.; Eikeler, D. Drug Dev Ind Pharm 2000, 26, 1239.
12. Marvola, M.; Aito, H.; Ponto, P.; Kanniksoki, A.; Nykanen, S.; Kokkonen, P. Drug Dev Ind Pharm 1987, 13, 1593.
13. Yang, L.; Chu, J. S.; Fix, J. A. Int J Pharm 2002, 235, 1.
14. Ojha, A. K.; Debabrata, M.; Krishnendu, C.; Subhas, M.; Roy, K.; Kaushik, G.; Islam, S. Carbohydr Res 2008, 343, 1222.
15. Wilson, C.; Washington, N.; Greaves, I.; Kamali, F.; Rees, J.; Sempik, A.; Lampard, J. Int J Pharm 1989, 50, 155.
16. Toti, U. S.; Aminabhavi, M. T. J Control Release 2004, 95, 567.
17. Durcilene, A. S.; Regina, C. M. P.; Judith, P. A. F. Eur Polym J 2007, 43, 2620.
18. Krishnaiah, Y. S. R.; Veer Raju, P.; Dinesh Kumar, B.; Bhaskar, P.; Satyanarayana, V. J Control Release 2001, 77, 87.
19. Mundargi, R. C.; Patil, S. A.; Agnihotri, S. A.; Aminabhavi, T. M. Drug Dev Ind Pharm 2007, 33, 255.
20. Mura, P.; Maestrelli, F.; Cirri, M.; Rodriguez, M. L. G.; Rabasco Alvarez, A. M. J Drug Targ 2003, 11, 365.
21. Wakerly, Z.; Fell, J. T.; Attwood, D.; Parkins, D. A. Int J Pharm 1996, 129, 73.
22. Ritger, P.; Peppas, N. J Control Release 1987, 5, 37.