Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2013; 5(4): 133-140

Research Article ISSN 0975-248X

Formulation Optimization for Colon Targeted Delivery of Katira Gum Matrix Tablets Containing Azathioprine

Priti Girotra, Shailendra Kumar Singh*

Department of Pharmaceutical Sciences, G. J. University of Sci. & Tech., Hisar-125001, India

ABSTRACT

Katira gum, a natural polysaccharide resembles guar gum in chemical composition and is susceptible to degradation by colonic microflora. Azathioprine is used in the treatment of inflammatory bowel disease, ulcerative colitis and Crohn's disease. Present investigation was undertaken to design Katira gum oral colon targeted matrix tablets containing azathioprine, in order to maximize its therapeutic efficacy. Central composite design (software design expert v. 8.0.7.1) was employed to optimize the formulation for maximum drug release in colon. The optimized batch tablets obtained were further coated by Eudragit S100 to minimize the drug release in upper part of GIT. The uncoated matrix tablets released around 20-50% of the drug in the physiological environment of stomach and small intestine but released all its contents in the colon. The coated optimized formulation exhibited a release of only 5-35% in stomach and small intestine and almost all drug in simulated colonic dissolution media. The drug release kinetics followed Korsmeyer-Peppas model, indicating the drug release mechanism through combined diffusion and erosion mechanism. The results suggested that Katira gum can be a good carrier for colon targeting owing to its moderate viscosity, biocompatibility and biodegradability which could be successfully degraded by the microbial flora in the colon.

Keywords: Azathioprine, Katira gum, central composite design, colon targeting, matrix tablets.

INTRODUCTION

Targeted delivery to the colon holds promise for direct and more effective delivery of therapeutic agents for the local treatment of gastrointestinal diseases such as colon cancer, irritable bowel syndrome, Crohn's disease and for systemic delivery of proteins and peptides. A colonic drug delivery system is anticipated to safeguard the drug release during its transit in the upper gastrointestinal tract (GIT) and permit its release only in the colon.

Azathioprine, an immunosuppressive antimetabolite, has been implicated in the treatment of inflammatory bowel disease [1], ulcerative colitis [2] and Crohn's disease. [3] The bioavailability of this drug upon oral ingestion is limited to an extent of 41-50%.

A large number of bacterial enzymes, present in the colon, fulfill their energy needs by fermenting endogenous and exogenous substrates such as carbohydrates and proteins, thus causing their assimilation. [4] Polysaccharides, commonly incorporated in pharmaceutical preparations, can be used as an alternative substrate for the bacterial enzymes present in the colon. The enzymes act on the matrices of polysaccharides or the coatings and release the drug from the dosage form. Katira gum, a plant exudate, is one such natural polysaccharide obtained from Cochlospermum species.

Amongst the twenty available species of Cochlospermum, *Cochlospermum religiosum* is the only species that is found in India. It consists of the repeating units of d-galactose, d-galacturonic acid and l-rhamnose in a molar ratio of 2:1:3. [5] Extensive research work has already been carried on other natural polysaccharides such as guar gum [6] and xanthan gum [7], although very little literature is available on Katira gum as it is also liable for colonic degradation by microbial flora.

The present study was therefore undertaken to evaluate the therapeutic potential of Katira gum in colon targeting of model drug Azathioprine with formulation optimization using central composite design (CCD) for the quantification of effect of formulation variables on the performance of dosage form.

MATERIALS AND METHODS

Materials: Azathioprine was generously gifted by Neon Laboratories Ltd. (Mumbai, India) and RPG Life Sciences Ltd. (Mumbai, India). All the chemicals utilized were of suitable analytical grade and used as and when required. Katira Gum was procured from local vendor at Hisar (Haryana) and authenticated vide reference no. NISCAIR/RHMD/Consult/-2011-12/1835/135.

***Corresponding author: Dr. Shailendra Kumar Singh,** Professor (Pharmaceutics), Department of Pharmaceutical Sciences, G. J. University of Sci. & Tech., Hisar-125001, India; **Tel.:** +91-9416473355; **Fax:** +91-1662-276240; **E-mail:** sksingh_gju@rediffmail.com

Animals: Six Wistar Rats (150-200 g) were procured from the Disease-Free Small Animal House, LLRUVS, Hisar, Haryana. All these animals received proper human care. The experimental protocol was approved by the Institutional Animals Ethics Committee (IAEC) (Registration number 0436).

Characterization of Azathioprine

Fourier Transform Infrared (FTIR) Analysis

The pure drug Azathioprine was subjected to FT-IR spectroscopy in a Fourier-Transform Infrared Spectrophotometer (FTIR Spectrophotometer Perkin-Elmer BX II) in the range of 4000-500 cm^{-1} using KBr pellet method.

Differential Scanning Calorimetric (DSC) Analysis

DSC thermogram of Azathioprine was recorded using Differential scanning calorimeter (Q10, TA Systems, USA). About 5 mg of samples were crimped in a standard aluminium pan and heated in a temperature range of 25 to 250°C at the heating rate of 10°C per minute in nitrogen atmosphere (flow rate, 20 mL/min). [8]

Characterization of Katira Gum

In addition to the FTIR and DSC analysis, Katira Gum was also characterized by the measurement of pH and viscosity of its 1% w/v solution. The swelling capacity of Katira gum was also determined, by placing 1g gum in 20 mL water in a measuring cylinder for 24 hours. Additional 80 mL water was added in the cylinder after 2 hours. The swelling index of the gum was obtained using the formula:

$$S.I = (V_2 - V_1)/ V_1$$

Where, S.I= Swelling Index; V_1= Initial volume of gum; V_2= Volume of hydrated gum

Experimental Design: In the present study, drug:polymer ratio (azathioprine:katira gum ratio), concentration of matrix forming polymer (methocel K15) and intragranular binder (potato starch) were used as independent variables at three levels (-1,0,+1) as formulation factors and the centre points (0,0,0) were studied in quintuplicate. The software Design Expert v 8.0.7.1 was employed for the generation and evaluation of central composite statistical experimental design, as shown in Table 1. Corresponding to the levels, -1, 0, +1, the amount of Katira gum used was 25, 50 and 75 mg respectively. Similarly, an amount of 5, 10, 15 mg were used respectively in correspondence to the levels -1, 0, +1 for both methocel K15 and potato starch.

Preparation of matrix tablets of Azathioprine: The tablets were prepared using wet granulation method, in a Cadmach single punch tablet compression machine. [9] Potato starch was incorporated as an intragranular binder and soluble starch as an extragranular binder.

Determination of Flow properties of granules: Granules were evaluated for their bulk and tapped density, Carr's index and Hausner's ratio, which are essential to be determined, so as to avoid any trouble in their compression.

Evaluation of matrix tablets: The physical parameters such as thickness, weight variation, hardness and friability were assessed in the prepared matrix tablets. To obtain the swelling index, accurately weighed tablets were placed in a petri dish containing 20 ml of phosphate buffer (pH 7.4) at room temperature. The tablets were periodically removed, blotted with blotting paper and again weighed. The swelling index was calculated using the equation:

$$\text{Swelling index} = (W_t - W_0)/W_0$$

where, W_t= Weight of tablet at time t; W_0= Weight of tablet at time t=0

***In-vitro* drug release study of matrix tablets of Azathioprine:** The *in-vitro* dissolution studies were carried out in USP-II dissolution apparatus at a stirring speed of 50 rpm in 900 mL of dissolution media maintained at 37±0.5°C. To mimic the GIT transit, the dissolution was carried in different bio-relevant media representing pH of particular anatomical region. For mimicking the gastric fluid in stomach, the dissolution was performed in 0.1 N HCl (pH=1.2) for two hours, in phosphate buffer (pH=6.8) to simulate the small intestinal fluid for three hours and for another three hours in phosphate buffer (pH=7.4), simulating the colonic environment. Sample aliquots withdrawn at specific time intervals, were analyzed at 280 nm using UV-Visible Double beam Spectrophotometer (Model-Systronics 2203) against suitable blank.

Drug release studies of the optimized batch matrix tablets in the presence of rat caecal content: The susceptibility of the tablets to undergo degradation in the presence of colonic bacteria was assessed by conducting the drug release studies in presence of rat caecal content. [10] Six Wistar rats (weighing 150-200 g) maintained on normal diet were fed with Katira gum (orally 1% w/w) for 15 days in order to induce the microflora and enzymes. Rat caecal content was removed and a 4% w/v dispersion in phosphate buffer (pH=6.8), was prepared. [11] It was used as the dissolution medium and conditions to mimic the anaerobic environment of colonic microflora were maintained by bubbling CO_2 after every half an hour and protected from light. The samples, withdrawn at specific time intervals for 24 hours, after suitable dilution, were analyzed at 280 nm using UV-Visible spectrophotometer.

Eudragit S100 coating of tablets for pH dependent release: For minimizing drug release in upper GIT, i.e. in stomach and small intestine, Eudragit S100 was selected as the pH dependent coating polymer. A 10% w/v Eudragit S100 coating solution was prepared using isopropyl alcohol (IPA) containing 3% plasticizer- Polyethylene glycol 400 (PEG 400). [12] The tablets of the optimized batch were coated using this solution by dip coating method.

Table 1: Experimental design (using design expert v 8.0.7.1)

Standard	Run	Drug:Polymer ratio	Conc. of matrix forming polymer	Conc. of intragranular binder
20	1	0.00	0.00	0.00
13	2	0.00	0.00	-1.68
10	3	1.68	0.00	0.00
3	4	-1.00	1.00	-1.00
7	5	-1.00	1.00	1.00
16	6	0.00	0.00	0.00
17	7	0.00	0.00	0.00
18	8	0.00	0.00	0.00
12	9	0.00	1.68	0.00
2	10	1.00	-1.00	-1.00
19	11	0.00	0.00	0.00
15	12	0.00	0.00	0.00
1	13	-1.00	-1.00	-1.00
9	14	-1.68	0.00	0.00
6	15	1.00	-1.00	1.00
4	16	1.00	1.00	-1.00
5	17	-1.00	-1.00	1.00
11	18	0.00	-1.68	0.00
14	19	0.00	0.00	1.68
8	20	1.00	1.00	1.00

Table 2: Flow properties of granules

Formulation Code	Bulk density	Tapped density	Carr's Compressibility index	Hausner's ratio
F1	0.60	0.69	13.04	1.28
F2	0.63	0.74	14.86	1.17
F3	0.62	0.72	13.88	1.16
F4	0.59	0.70	15.71	1.18
F5	0.57	0.71	19.71	1.22
F6	0.59	0.74	20.27	1.25
F7	0.55	0.67	17.91	1.21
F8	0.61	0.70	12.85	1.14
F9	0.54	0.68	20.58	1.25
F10	0.57	0.68	16.17	1.19
F11	0.61	0.69	11.59	1.13
F12	0.60	0.70	14.28	1.16
F13	0.61	0.75	18.66	1.22
F14	0.60	0.73	17.80	1.21
F15	0.58	0.69	15.94	1.18
F16	0.63	0.74	14.86	1.17
F17	0.62	0.72	13.88	1.16
F18	0.64	0.75	14.66	1.17
F19	0.57	0.70	18.57	1.22
F20	0.66	0.77	14.28	1.16

Table 3: Evaluation tests for matrix tablets

Formulation code	Weight variation	Thickness (mm)	Hardness (kg/cm^2)	Friability (%)
F1	149.48 ± 4.05	0.4±0.12	5.28 ± 0.14	0.6
F2	170.52 ± 5.36	0.5±0.15	5.24 ± 0.24	0.8
F3	157.73 ± 6.56	0.4±0.11	5.88 ±0.21	0.9
F4	171.19 ±7.33	0.3±0.18	5.8 ±0.2	0.9
F5	199.61 ± 5.96	0.6±0.2	5.92 ±0.21	0.6
F6	180.94 ± 7.38	0.4±0.21	5.18 ± 0.17	1.0
F7	166.87 ±6.43	0.6±0.13	5.08 ±0.07	0.6
F8	168.45 ±6.30	0.5±0.15	5.5 ± 0.14	0.7
F9	245.91 ± 2.54	0.3±0.16	5.14 ±0.10	0.5
F10	183.90 ± 6.73	0.5±0.08	5.4 ±0.17	0.8
F11	139.97 ± 5.73	0.5±0.12	5.66 ± 0.14	0.5
F12	203.17 ± 7.11	0.3±0.16	5.56 ± 0.18	0.7
F13	134.43 ± 6.64	0.2±0.18	3.08 ± 0.17	1.2
F14	117.35 ± 7.45	0.2±0.25	3.92 ± 0.28	1.4
F15	196.77 ± 5.77	0.6±0.22	4.98 ± 0.23	0.7
F16	147.53 ± 4.72	0.4±0.14	5.48 ± 0.20	0.6
F17	157.29 ± 4.74	0.4±0.18	5.52 ± 0.11	0.7
F18	160.03 ±5.79	0.3±0.11	5.2 ± 0.14	0.9
F19	166.01 ± 4.87	0.6±0.21	5.02 ± 0.11	0.8
F20	147.92 ± 3.88	0.5±0.09	4.74 ± 0.10	0.6

Drug Release kinetics: Data obtained from the *in-vitro* drug release studies of matrix tablets of optimized formulation were evaluated using various kinetic models such as zero order, first order, Higuchi model and Korsmeyer-Peppas model.

RESULTS

Characterization of drug and polymer

FTIR spectrophotometric analysis: Figure 1 represents the FTIR spectrum of Azathioprine, Katira gum and optimized batch matrix tablets. It was carried out to evaluate the possibility of interaction between the drug and the polymers used. Azathioprine depicted a number of characteristic peaks at 916, 1228, 1382, 1529, 1589, 3115 cm^{-1} which may be attributed to the C-H deformation, C-N stretch, CH_3 bend, C-NO_2 stretch, C=N and N-H stretch respectively. The most important peak obtained in the FTIR spectrum of Katira gum was found to be at 1332 cm^{-1} which may be ascribed to CH_3 bend. The spectra of Katira gum optimized batch matrix tablets containing Azathioprine showed all these characteristic peaks of the drug and polymer indicating the absence of shifting or splitting in absorption peaks. Thus, it may be concluded that the chemical interaction between drug and other excipients did not take place during processing.

Differential Scanning Calorimetric analysis: A comparison of the thermograms of pure drug, polymer and formulation has been displayed in Figure 2. In case of DSC thermogram of azathioprine, a sharp endothermic peak was observed at 268.77°C and an exothermic peak was observed at 263.39°C. Thermogram of Katira gum showed a broad peak at 110.78°C; whereas, the optimized batch exhibited a broad peak at 100.64°C and a sharp peak at 262.08 and 297.65°C. In the thermogram of matrix tablet the peaks for azathioprine was observed, although its intensity was less.

Determination of pH of Katira Gum (1% w/v): The pH of 1% w/v Katira gum was found to be 6.4, indicating slightly acidic nature of Katira Gum.

Determination of viscosity of Katira Gum: The viscosity of 1% w/v solution, measured at 25±1°C using Brookfield DV-E viscometer, USA with spindle number 02, was found to be 66.4 cP and 62 cP at the speed of 100 and 60 rpm of the spindle respectively. Katira gum, in contact with water, forms a thick colloidal hydrophilic gel which on incorporation in the tablets, is responsible for the exhibition of its release retardant property. Drug release from such hydrophilic gels is expected by diffusion of drug solution through gel network.

Determination of swelling capacity of Katira gum: Swelling index of gum Katira was observed to be 26.66 showing excellent swelling capacity by imbibition of water. High swelling capacity suggests its possible use as tablet binder, disintegrant etc.

Determination of flow properties of granules: Bulk density and tapped density were found ranging from 0.54 to 0.66 and 0.67 to 0.77 respectively. Carr's compressibility index (C.I) and Hausner's ratio was found to fall in the range of 11.59 to 20.58 and 1.13 to 1.28 respectively (Table 2). These results represent quite good flow properties of the granules.

Evaluation of formulated matrix tablets of azathioprine: Since Katira Gum was found to have poor flow properties and was to be incorporated in the matrix tablets in a larger proportion, the tablets were prepared by wet granulation technique using starch as a binder. The physical parameters, such as thickness, weight variation, friability and hardness were found to be within the permissible limits [13] for tablets (as depicted in Table 3). Table 4 highlights the swelling index of formulated matrix tablets. The degree of swelling of the prepared matrix tablets was observed to depend on the amount of Katira gum and HPMC.

***In-vitro* dissolution study of formulated matrix tablets in different bio-relevant dissolution media:** Katira Gum matrix tablets released about 20-50% of the drug Azathioprine in the physiological environment of stomach and small intestine (pH= 1.2 and 6.4) as depicted in figure 3 and figure 4 respectively. The matrix tablets released majority of its drug content in the physiological environment of colon (pH=7.4) as portrayed in figure 5. This indicates that Katira gum used in the formulation was successfully degraded by microbes present in the colon.

Solution of Numerical Optimization (using Design Expert v 8.0.7.1): The goal of optimization was to minimize the drug release in 2 hours and 5 hours (i.e. in stomach and small intestine) and maximizing the drug release in 8 hours (i.e. in the colon) as shown in Table 5. Based on the desirability approach for optimization, the solution indicated a drug:polymer ratio at +2 level, concentration of matrix forming polymer at -0.97 level and concentration of

intragranular binder at -2 level, yielding 1.8% and 12.98% drug release in 2 and 5 hours respectively with entire drug release in 8 hours with desirability of 0.878. Cube plots for easier visualization of effect of three independent variables on response were generated (Figure 6), which indicated that concentration of intragranular binder be minimized to -2 level and matrix forming polymer to -0.27 level with minimum drug:polymer ratio for maximum desirability.

Table 4: Swelling index of tablets

Time (hr)	F1	F2	F3	F4	F5
0.5	260	155.1766	237.0006	94.23559	157.6609
1	263.7061	211.2667	256.051	163.7218	197.8039
1.5	Tablet disintegrated	270.7674	267.7475	172.8697	232.7886
2	-	483.0085	281.1812	240.3509	328.5495
4	-	563.8246	288.1297	399.0602	543.5138
6	-	594.2753	325.4198	424.3108	609.9081
8	-	597.9294	329.8205	429.1353	625.2809
10	-	600.3654	338.2745	461.0276	604.8008
12	-	607.1864	342.5594	465.3509	608.6823
Time (hr)	**F6**	**F7**	**F8**	**F9**	**F10**
0.5	128.9692	419.0801	131.0667	98.36683	182.4401
1	202.0735	474.9574	191.4535	117.0854	224.9145
1.5	251.5995	496.2521	248.2221	167.9229	354.39
2	238.3294	479.2164	350.8422	183.3333	589.8518
4	277.6066	513.2879	423.2689	201.0469	699.3158
6	445.8531	536.0023	464.1298	280.8626	669.6693
8	557.9976	544.1794	468.1847	308.5846	680.4447
10	562.3223	490.5735	472.5515	318.4255	684.2075
12	566.4692	496.8768	477.4173	322.9481	694.4698
Time (hr)	**F11**	**F12**	**F13**	**F14**	**F15**
0.5	283.0145	107.7756	251.7526	376.5144	119.4476
1	294.1086	150.5906	323.299	374.1845	162.5432
1.5	295.4858	175.2953	271.5464	396.5517	176.6398
2	298.3168	186.3189	264.811	366.6356	201.611
4	318.6687	232.3819	288.3849	390.028	230.7825
6	337.7965	348.622	270.1718	401.8639	327.2152
8	356.3887	360.3346	272.9897	409.972	332.5662
10	370.6963	385.1378	278.8316	425.3495	344.4764
12	380.3366	390.6496	287.7663	436.8127	350.6329
Time (hr)	**F16**	**F17**	**F18**	**F19**	**F20**
0.5	191.0828	188.9026	190.0131	228.1081	72.10702
1	299.08	262.2072	448.0946	452.1922	91.23746
1.5	325.0531	277.0037	512.8121	413.2132	108.5619
2	304.034	290.7522	557.0302	478.3784	128.6957
4	311.6773	298.1504	570.1708	471.1712	140.8696
6	325.1946	320.9001	570.1708	494.955	132.3746
8	329.3701	325.6473	580.3548	498.3784	141.3378
10	335.8811	329.6547	596.452	508.4084	150.7023
12	340.9059	335.8816	606.1761	516.2162	155.5184

Study of release kinetics of optimized batch: Zero order was found to be the best fit mathematical model for the uncoated matrix tablets, suggesting that the drug released by diffusion through the polymeric gel network. The drug release kinetics for the coated matrix tablets was observed to be best described by Korsmeyer-Peppas, indicating the drug release through a combination of diffusion as well as erosion mechanism.

***In-vitro* dissolution study of optimized batch matrix tablets:** During the *in-vitro* dissolution of the optimized batch in rat caecal medium, the tablet remained intact and slightly swollen for about 5 hours of the dissolution study. It was examined that with the amount of Katira gum increased in the formulation, percentage release of drug decreased proportionally and vice-versa. On exposure to dissolution fluids, the gum gets hydrated and forms a viscous gel layer around the tablet, slowing down further seeping of the dissolution fluids towards the core of the tablets. The coating of the tablets with Eudragit S100 significantly reduced the release of drug (5-35%) in stomach and small intestine, thus allowing a successful targeting of the formulation to the colon.

Table 5: Solution of Numerical Optimization

Constraints name	Goal	Lower limit	Upper limit	Weight	Importance
A:Drug: Polymer ratio	Is in range	-2	2	1	2
B: Conc. of matrix forming polymer	Is in range	-2	2	1	1
C: Conc. of intragranular binder	Is in range	-2	2	1	1
Cumulative drug release in 2 hrs	Minimize	1	12	1	3
Cumulative drug release in 5 hrs	Minimize	8	20	1	2
Cumulative drug release in 8 hrs	Maximize	95	100	1	5

No.	Drug: polymer ratio	Conc. of matrix forming polymer	Conc. of intra-granular binder	% Cum-ulative drug release in 2 hrs	% Cum-ulative drug release in 5 hrs	% Cum-ulative drug release in 8 hrs	Desir-ability	1 solution found
1	2.00	-0.97	-2.00	1.80205	12.9843	100	0.878	Selected

DISCUSSION

Azathioprine matrix tablets using Katira gum were prepared using Cadmach Single Punch Tablet Machine with varying ratio of drug:polymer, matrix forming polymer and intragranular binder. The precompression characteristics (Bulk density, Tapped density, Carr's Compressibility Index and Hausner's ratio) were evaluated. These parameters indicated good flow properties of the granules.

The matrix tablets were successfully formulated by wet granulation technique and were characterized by FTIR and DSC studies. The FTIR spectrum of pure azathioprine drug and pure polymer (Katira gum) were compared with the spectrum of optimized batch of azathioprine matrix tablets prepared using Katira gum. The presences of all characteristic peaks of Azathioprine and Katira gum in the IR spectrum of matrix tablets indicated absence of chemical interaction between drug and other excipients during processing.

DSC thermograms of pure drug Azathioprine, pure polymer Katira gum and optimized batch have been depicted in the overlay shown in figure 2. Peak intensity of azathioprine was less as compared to pure drug indicating the presence of azathioprine in less crystalline form in the tablet which may be attributed to wet granulation during processing which might have converted the drug from more crystalline form to amorphous form. All other exothermic and endothermic peaks in the formulation were similar to that in the thermograms of pure drug and pure polymer, thus proving

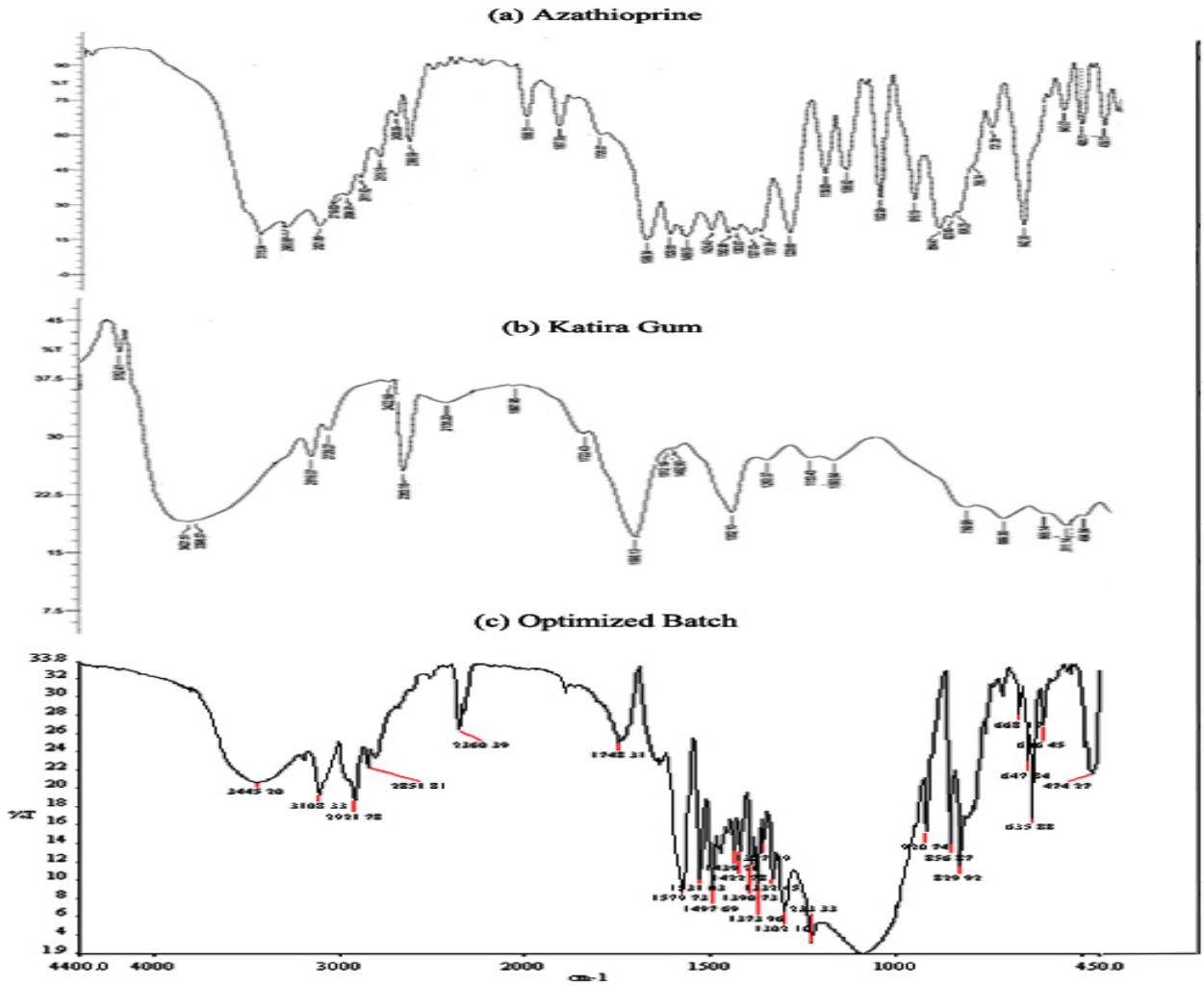

Fig. 1: FTIR spectrum of (a) Azathioprine (b) Katira Gum (c) Optimized batch matrix tablets

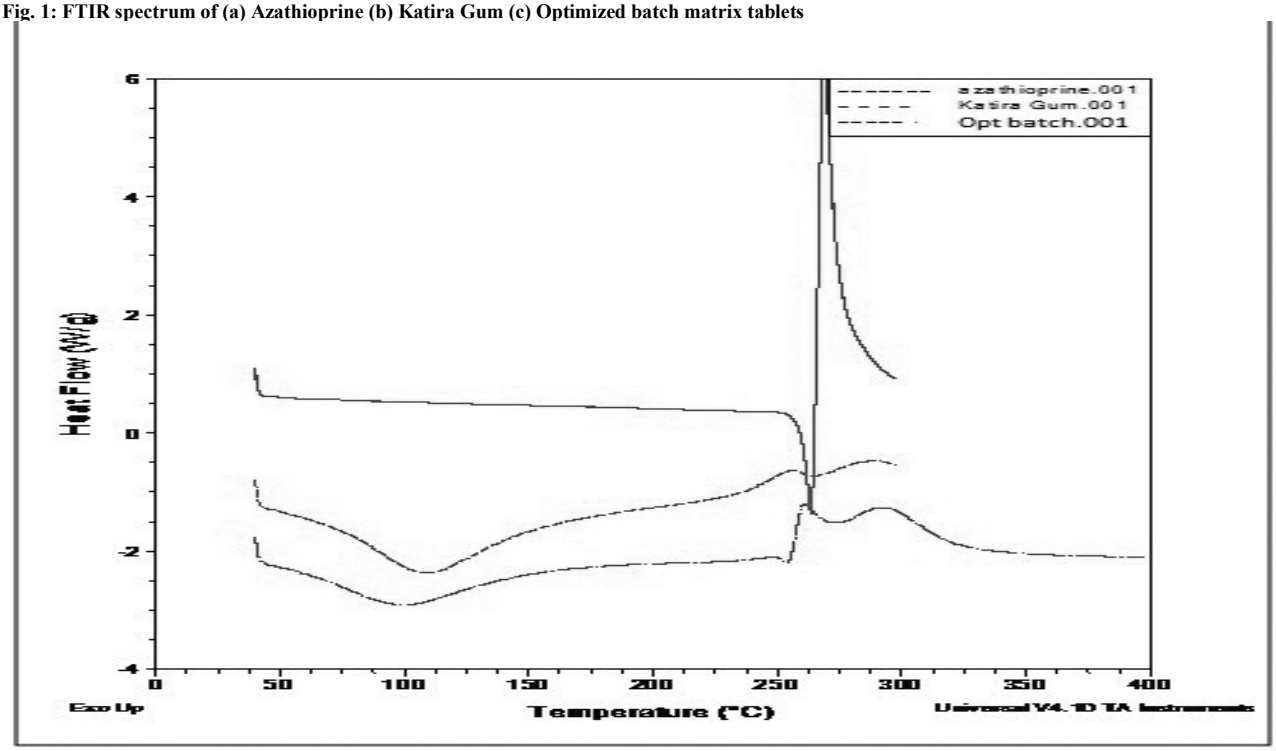

Fig. 2: Overlay of DSC analysis of azathioprine, Katira gum and optimized batch formulation

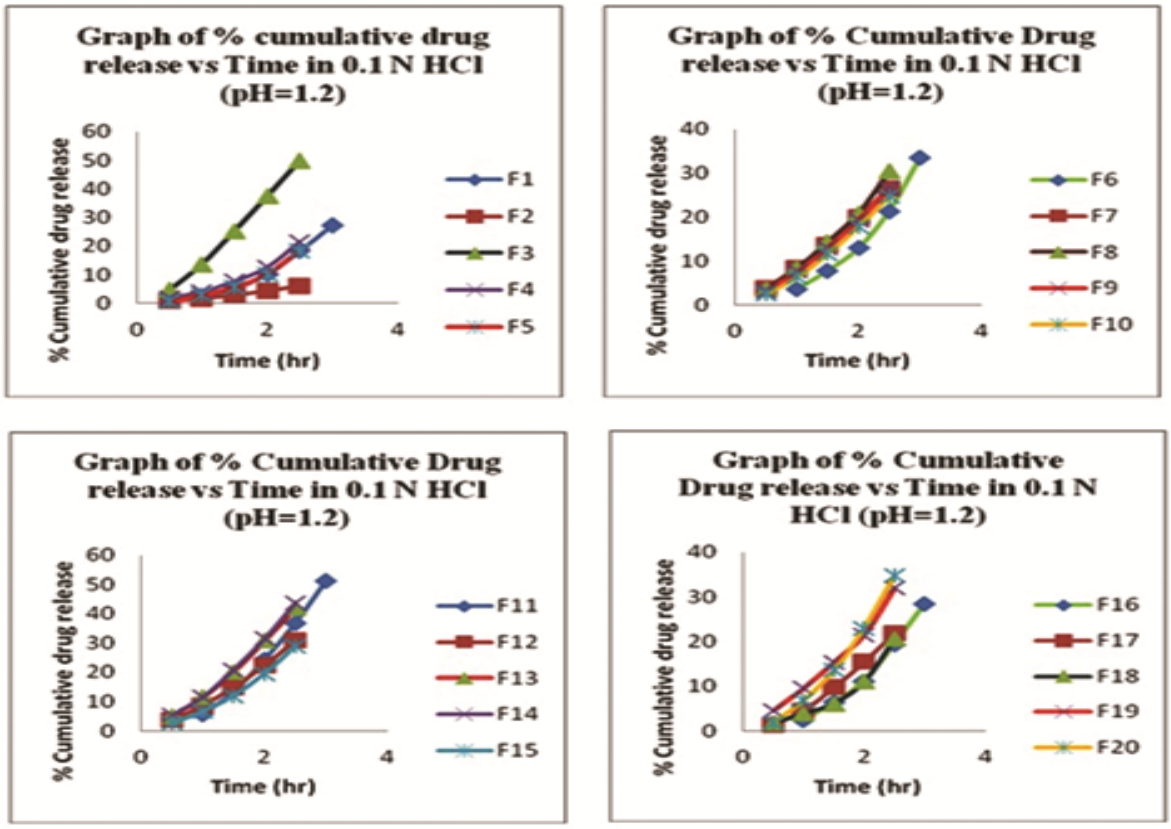

Fig. 3: ***In-vitro*** **dissolution study of prepared matrix tablets in dissolution medium having pH=1.2**

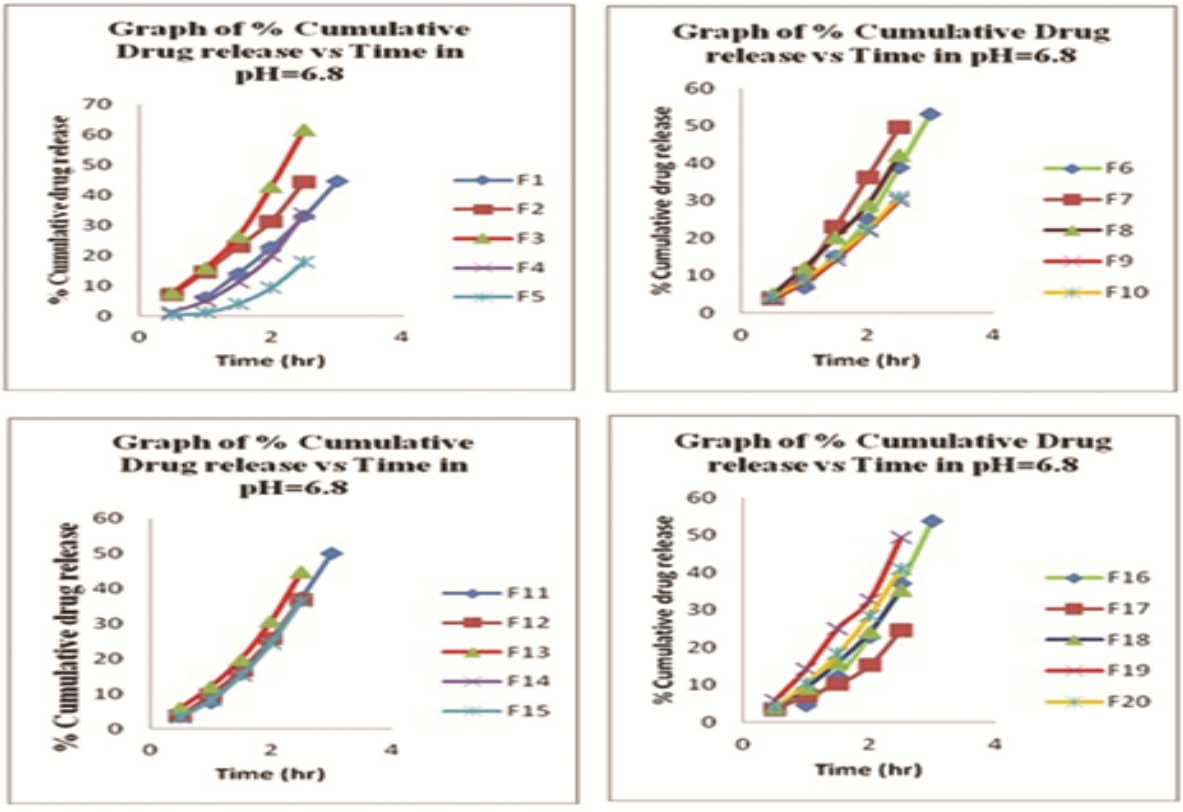

Fig. 4: ***In-vitro*** **dissolution study of prepared matrix tablets in dissolution medium having pH=6.8**

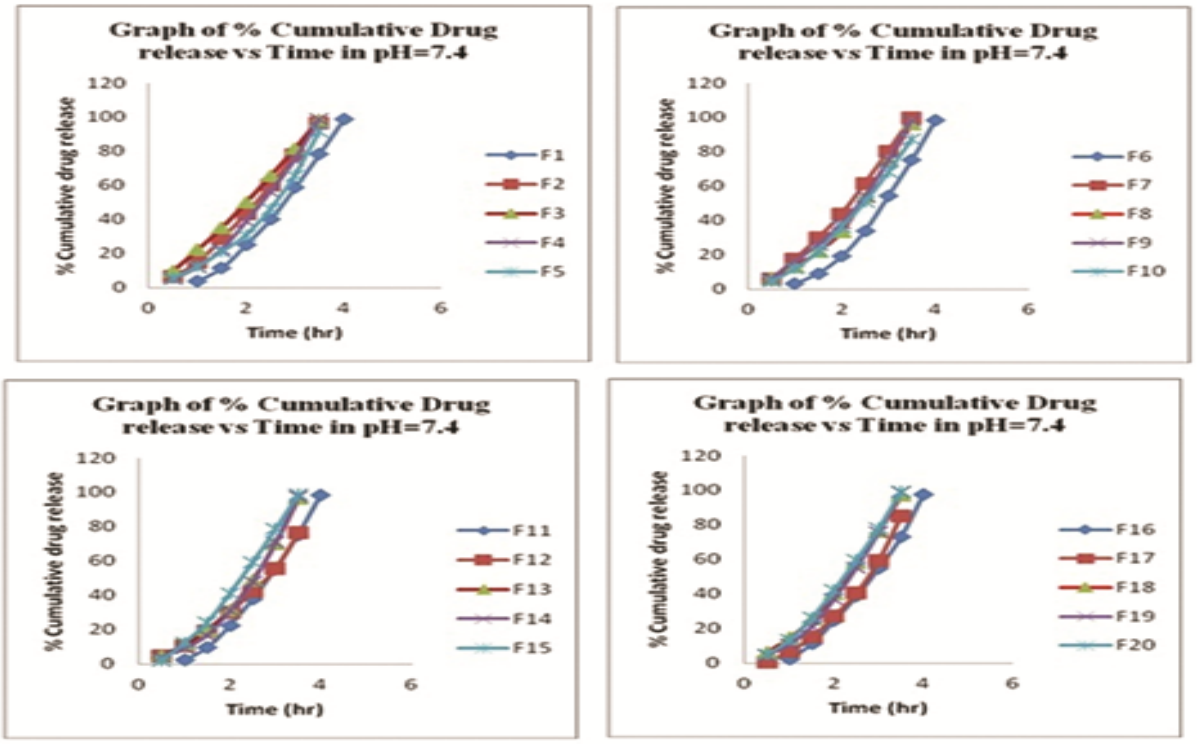

Fig. 5: *In-vitro* dissolution study of prepared matrix tablets in dissolution medium having pH=7.4

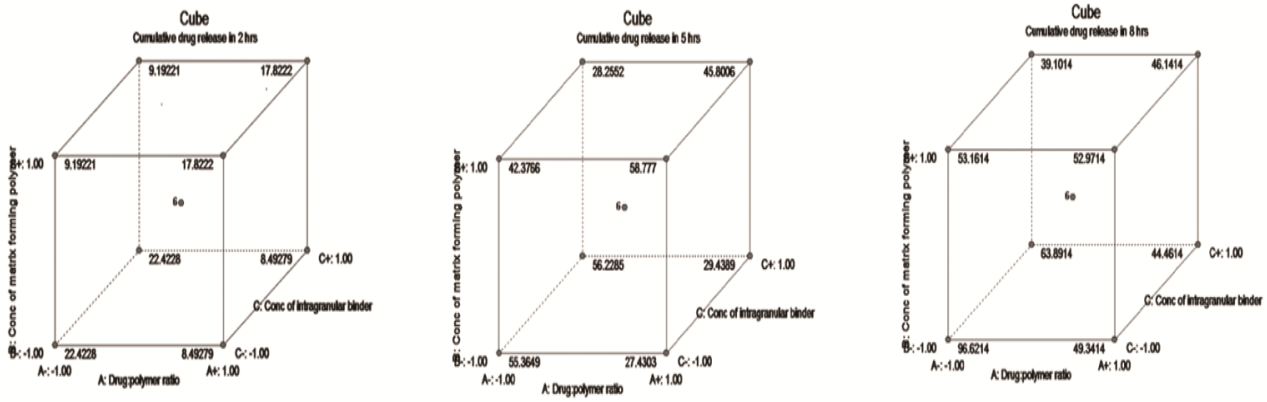

Fig. 6: Model graphs of cumulative drug release in 2 hrs, 5 hrs and 8 hrs

the absence of any chemical interaction between the drug and other excipients.

The tablets were evaluated for various physical tests such as weight variation, thickness, hardness and friability, which conformed to the prescribed limits. The effect of various formulation variables like drug:polymer ratio, the amount of matrix forming polymer and the amount of intragranular binder was evaluated.

The *in-vitro* dissolution studies of the prepared tablets, carried out in different bio-relevant media (pH=1.2; pH=6.8 and pH=7.4) showed the maximum percentage drug release in the colonic conditions but a small percentage of the drug was also observed to be released in the stomach and small intestinal pH. The criteria chosen to optimize the tablet formulation constraints viz. drug:polymer ratio, amount of matrix forming polymer (methocel K15) and intragranular binder (potato starch) were percent drug release in 2 hrs, 5 hrs and 8 hrs. The desirability function in optimizing the formulation with an importance value of 5 was to minimize the drug release in 2 hrs and 5 hrs; and maximize the drug release in 8 hrs, i.e. the tablet must release all its drug contents in colon. The level of constraints that guarantee such a response, obtained using the software Design Expert v 8.0.7.1, were drug:polymer ratio (2), methocel K15 (-0.97) and potato starch (-2.00). The optimized batch formulation was prepared and was selected for the enteric coating of matrix tablets using Eudragit S100 to minimize the drug release in upper GIT and for the evaluation of the percentage drug release in the presence of rat caecal contents. It was observed that the tablet remained intact in the stomach and small intestine but released all its drug content (~100%) in the microbial environment, mimicking the colon. Zero order drug release was found to be the most suitable best fit for uncoated matrix tablets whereas the release kinetics of coated

matrix tablets was best described by Korsmeyer-Peppas model, suggesting the drug to be released by diffusion from polymeric gel network as well as by erosion mechanism from the coated tablets.

Thus, the present study unraveled the effect of formulation variables on the drug release from the matrix tablets in different physiological environment of stomach, small intestine and colon. The results of this investigation clearly demonstrate that the optimized formulation of matrix tablets using Katira gum is capable of successfully targeting the drug Azathioprine to the colon by the combination of pH dependent and microbially triggered approach for the efficient treatment of inflammatory bowel disease, Crohn's disease and ulcerative colitis. The present research work offers immense scope for further exploitation of Katira gum in future for targeting of other drugs to the colon.

ACKNOWLEDGEMENTS

The authors greatly acknowledge Neon Laboratories Ltd. (Mumbai, India) and RPG Life Sciences Ltd. (Mumbai, India) for providing gift sample of azathioprine. The authors also express their sincere thanks to All India Council of Technical Education, New Delhi for financial support.

REFERENCES

1. Dayharsh GA, Loftus EV, Sandborn WJ, Tremaine WJ, Zinsmeister AR, Witziq TE, Macon WR, Burgart LJ. Epstein-Barr virus-positive lymphoma in patients with imflammatory bowel disease treated with azathioprine or 6-mercaptopurine. Gastroenterol. 2002; 122:72-77.
2. Ardizzone S, Molteni P, Imbesi V, Bollani S, Bianchi Porro G, Molteni F. Azathioprine in steroid-resistant and steroid-dependent ulcerative colitis. J Clin Gastroenterol. 1997; 25:330-333.
3. Lewis JD, Schwartz JS, Lichtenstein GR. Azathioprine for maintenance of remission in Crohn's disease: Benefits outweigh the risk of lymphoma. Gastroenterol. 2000; 118:1018-1024.
4. Van den Mooter G, Kinget R. Oral colon-specific drug delivery- A review. Drug Deliv. 1995; 2:81-93.
5. Ojha AK, Maiti D, Chandra K, Mondal S, Das D, Roy SK, Ghosh K, Islam SS. Structural assignment of a heteropolysaccharide isolated from the gum of Cochlospermum religiosum (Katira Gum). Carbohydr Res. 2008; 343:1222-1231.
6. Chaurasia M, Chourasia MK, Jain NK, Jain A, Soni V, Gupta Y, Jain SK. Cross-linked guar gum microspheres: A viable approach for improved delivery of anticancer drugs for the treatment of colorectal cancer. AAPS Pharm Sci Tech. 2006; 7:E143-151.
7. Alvarez-Mancenido F, Landin M, Martinez-Pacheco R. Konjac glucomannan/xanthan gum enzyme sensitive binary mixtures for colonic drug delivery. Eur J Pharm Biopharm. 2008; 69:573-581.
8. Rajera R, Nagpal K, Singh SK, Mishra DN. Toxicological study of the Primaquine phosphate loaded chitosan nanoparticles in mice. Int J Biol Macromol. 2013; 62:18-24.
9. Bharaniraja B, Kumar KJ, Prasad CM, Sen AK. Different approaches of Katira gum formulations for colon targeting. Int J Biol Macromol. 2011; 49:305-310.
10. Gauri B, Nagpal L, Singh SK. Formulation and Gamma Scintigraphic Evaluation of Colon targeted drug delivery systems of tinidazole in healthy human volunteers. J Pharm Biomed Sci. 2011; 7:1-9.
11. Sinha VR, Mittal BR, Kumria R. *In-vivo* evaluation of time and site of disintegration of polysaccharide tablet prepared for colon-specific drug delivery. Int J Pharm. 2005; 289:79-85.
12. Purushothaman M, Vijaya RJ. Formulation optimization and release kinetics of tinidazole matrix, compression and spray coated tablets: effect of organic acid on colon targeted drug delivery systems. JITPS. 2010; 1:18-32.
13. Banker GS, Anderson NR. The Theory and Practice of Industrial Pharmacy. Edn 3, Varghese Publishing House, Bombay, 1987, pp. 296-302.