Research Article

Formulation and Evaluation of Microbial Triggered Colon Specific Delivery of Azathioprine

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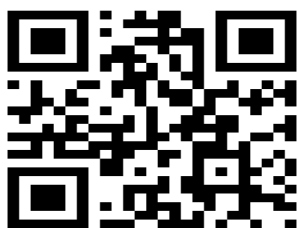Mahakal Institute of Pharmaceutical Studies,Ujjain (M.P).

ABSTRACT

Purpose: The Thioguanine derivative, Azathioprine (AZA) is a prodrug of 6-mercaptopurine that is further metabolized by various enzymes present in the liver and gut have proven efficacy in the treatment of inflammatory bowel disease (ulcerative colitis) in 2-2.5 mg/kg/day at 0,2 and 6 weeks than every 8 week and also may reduce the need for steroid treatment. In the present work sustained release formulation of azathioprine was developed with an objective to achieve colon specific drug delivery with reduced frequency of dosing, to minimize gastric side effect and thus to increase patient compliance.

Method: The six different tablet formulations were prepared by direct compression method using Guar gum (GG), and xanthan gum (XG). Tablets are evaluated for their physicochemical properties and in vitro drug release studies. Biodegradability studies of guar and xanthan gum was carried out in presence of 4% w/v RCC and galactomannase enzyme (0.1 mg/ml.) by viscosity measurement using Brookfield viscometer and significant decrease in viscosity was found with 4% RCC after 24 h incubation.

Result: In the rat caecal contents formulations shows enhanced drug release due to degradation of guar gum coat by colonic galactomannanase enzyme. The tablets containing guar (G1) released 70.07 %, at the end of 24th hour in rat caecal contents whereas, drug release from tablets containing a xanthan gum (X6) 79.06 % at the end of 24th hour.

Conclusion: From the results of this study it appears that, the proposed microbial triggered matrix tablet of Optimized X6 formulation azathioprine (500 mg) conventional tablet with better control of drug release for targeted drug delivery. In addition developed colon-specific drug delivery system (CDDS) was relatively inexpensive and easy to manufacture using other techniques.

Keywords: Azathioprine, bacterially triggered, matrix tablets, colon targetting

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Conflict of Interest: None declared

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INTRODUCTION

Most of the drugs when taken through oral route release their content in stomach and small intestine. Drugs are also metabolized through phenomenon called as first pass metabolism. So drugs are targeted particularly to colon to prevent first pass metabolism and release of drugs in stomach and small intestine. Site specific drug delivery to colon allows oral administration of such drugs which are normally inactivated in degradation of the drug in the upper part of gastrointestinal tract (GIT) which must be upper part of gastrointestinal tract.

Colonic drug delivery may be achieved by either oral or rectal administration. Rectal dosage forms (enemas and suppositories), are not always much effective due to high variability in the distribution of drug administered by this route.*3* The major obstacle with the delivery of drugs by oral route to the colon is the absorption and

overcome for successful colonic drug delivery (1).

The Thioguanine derivative, Azathioprine (AZA) is a prodrug of 6-mercaptopurine that is further metabolized by various enzymes present in the liver and gut have proven efficacy in the treatment of inflammatory bowel disease. Its parent drug 6-mercaptopurine (6-MP), and the closely related 6-thioguanine (6-TG), were originally developed for their anticancer properties, but thiopurines as a class are now more widely used for their anti-inflammatory and immunosuppressant effects. Azathioprine and 6-mercaptopurine may be effective for inducing remission in Crohn's disease among patients with chronically active disease. These drugs may reduce the need for steroid treatment and their use may therefore lead to a lower incidence of steroid related side effects (2).

Various approaches have been used for delivery of drugs to the colon *via* oral route, which include coating with pH-dependent polymers, design of time-release dosage forms and the utilisation of carriers that are degraded exclusively by the colonic bacteria (3). Every system has advantages as well as disadvantages. The poor site-specificity of pH-dependent systems, because of large variations in the pH of the gastrointestinal tract, is very well documented. The site-specificity of timed-release dosage forms is considered poor because of large variations in gastric emptying times and passage across the ileo-cecal junction (4). However, microflora-activated systems (microbially triggered delivery system) formulated making use of non-starch polysaccharides are highly promising because the polysaccharide remain undigested in the stomach and the small intestine and can only be degraded by the vast anaerobic microflora of the colon.

Both anaerobic and aerobic microorganisms inhabit the human gastrointestinal tract. The microflora of the small intestine is mainly aerobic, whereas in large intestine, it is mainly anaerobic and about 400 bacterial species have been reported in colon. Most bacteria inhabit the proximal part of large intestine, where energy source are greatest.

The upper part of GIT, i.e. the stomach and the duodenum has a microflora of less than 103–104 CFU/ml. The microflora of colon on the other side is in the range of 1011–1012 CFU/ml consisting mainly of anaerobic bacteria, e.g. *Bacteroides, Bifidobacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria*, etc

This vast microflora fulfils its energy needs by fermenting various types of substrates that have been left undigested in the small intestine, e.g, di and trisaccharides, polysaccharide etc. For this fermentation, the microflora produces a vast number of enzymes like glucuronidase, xylosidase, arabinosidase, galactosidase, nitroreductase, azoreductase, deaminase and urea dehydroxylase . Because of the presence of these biodegradable enzymes only in the colon, the use of bacterial degradable polymers for colon specific drug delivery seems to be a more site specific approach as compared to other approaches . Some studies were carried out on the basis of the activity of colonic bacteria on polysaccharide.

Among the various system developed for colon specific drug delivery, prodrug and polysaccharide based delivery system rely upon the enzymatic degradation in the colon, there by resulting in the drug release. The inharent bacterial flora present in the colon carries out this colon degradation. The enzyme trigger mechanism in such delivery systems makes them highly site specific. , natural polysaccharides, fall under the categories of "GRAS"(generally regarded as safe),thus resolving the general problems associated with safty(5). The polysaccharides for colonic drug delivery are also inexpensive, naturally occurring and abundantly available (6).

MATERIALS

Azathioprine was gift sample from RPG Lifescience limited, Mumbai, (Maharastra). Sodium chloride, Guar gum, Xanthan gum from Loba Pharmaceuticals, Gallactomannase enzyme from Aum enzyme, Barsad (Gujrat) and Microcrystalline cellulose, Magnesium stearate were Obtained from Signet Chemical Corporation, Mumbai.

ASSESSMENT OF BIODEGRADABILITY (7)

The enzymatic activities associated with microflora of colon can be used as a tool for colon specific drug delivery. In addition, colon has a longer retention time and appears to be highly responsive to agents that enhance the absorption of poorly absorbed drugs.

So before formulation, assessment of biodegradability of polysaccharides was carried out by viscosity measurement. Biodegradability of guar gum and xanthan gum was assessed by conducting viscosity measurement on guar gum and xanthan gum dispersion prepared in phosphate buffer pH 7.4, in presence of rat caecal contents and in galactomannase enzyme.

Studies in presence of galactomannase enzyme

0.5% w/v dispersion was prepared by dispersing guar gum and xanthan gum powder in pH 5.9

Sorensen phosphate buffer with Galactomannase enzyme (0.1 mg/ml) previously bubbled with CO_2 and allowed to hydrate for 24 h in stoppered conical flask maintaining CO_2 environment. After 24 h viscosity was measured at 20 rpm ate 37 °C using spindle no. 2 with Brookfield viscometer. The dispersion was incubated at 37 °C for 2 hours and again viscosity was determined at 37 °C. The incubation was continued for 24 h at 37 °C. After 24 h viscosity was determined at 37 °C, 20 rpm, CO_2 environment was maintained all through the experiment, which were done in triplicate.

Studies in absence of rat caecal contents
0.5% w/v dispersion was prepared by dispersing guar gum and xanthan gum in pH 7.4 phosphate buffers (PB) previously bubbled with CO_2. The dispersion was allowed to hydrate for 24 hours in stoppered conical flask maintaining CO_2 environment. After 24 h viscosity was determined at 20 rpm at 37 °C with spindle no. 2 using Brookfield viscometer. Then dispersion was incubated at 37°C for 2 h and again viscosity was determined at 37 °C. The incubation was continued for 24 h at 37 °C. After 24 h Viscosity was determined at 37 °C, 20 rpm and CO_2 environment was maintained through about the experiment. The viscosity measurements were done in triplicate.

Studies in presence of rat caecal
Contents Preparation of rat caecal content medium Albino rats weighing 150-200 g maintained on normal animal feed, (Gold Mohar rat feed, Hindustan Lever Ltd.) were used for the preparation of rat caecal content medium, without enzyme induction. Thirty minutes before commencement of studies, three rats were sacrificed. The abdomen was opened and the caecum isolated, legated at both ends, cut loose and immediately transferred to pH 7.4 phosphate buffer previously bubble with CO_2. The caecal bags were opened, the contents were individually weighed, mixed and suspended in pH 7.4 phosphate buffer to give required caecal dilution of 2% w/v. As the caecum is naturally anaerobic, all these operations were carried out in CO_2 environment. Similarly six rats were used for preparing a caecal dilution of 4% w/v.

The biodegradation study was carried out in the same way as in the absence of rat caecal contents except that, before incubation at 37°C, 5 ml phosphate buffer containing 2 % w/v and 4 % w/v rat caecal contents was added separately to the respective dispersed guar and xanthan gum. CO_2 environment was maintained during the

Biodegradation studies. The study was performed in triplicate.

The enzyme galactomannase induced in rat caecal fluid hydrolyze polymeric linkages in guar and xanthan gum. As shown in table 1, the degradation of polysaccharides is more in presence of 4% w/v rat caecal content (RCC) in presence of galactomannase enzyme as compared to 2% rat caecal content as evidenced by decreased viscosity in 4% w/v rat caecal content than 2% rat caecal content. The control samples have not shown any drastic changes in the viscosity values. Due to this result 4% w/v rat caecal content was selected for the drug release with pH 7.4 phosphate buffer.
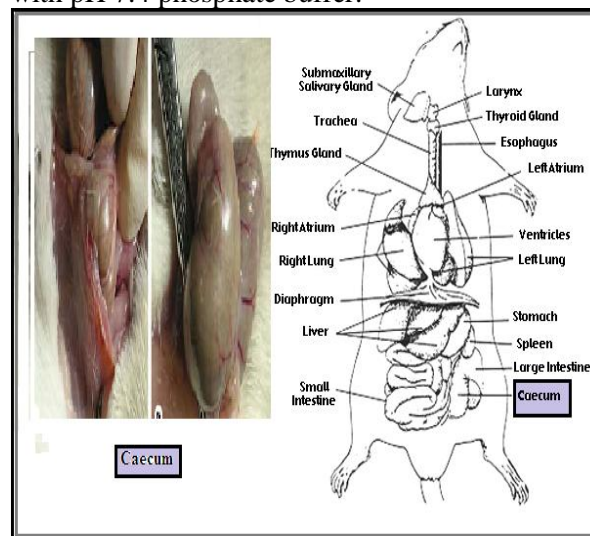

Figure 1: Position of caecum in rat

Viscosity	Sample without RCC (Control)	Sample with RCC (2% w/v)	Sample with RCC (4% w/v)	Sample with galactomanse Enzyme (0.1 mg/ml)
At 37 °C initial hydrated	1181	1161	1121	1161
At 37 °C after 2 h incubation	1181	1101	1041	1061
At 37 °C after 24 h incubation	1161	941	**661**	821

Table 1: Viscosity Measurement Studies in Presence and Absence of Rat Caecal Content and in Presence of Galactomannase Enzyme.

PREPARATION OF TABLET

The matrix tablets of Azathioprine were prepared by employing various polysaccharides like Guar gum, and Xanthan gum by direct compression method using 8 mm flat-faced punch of 10 stations Rimek compression machine. For the preparation of matrix tablets, the active ingredient was thoroughly mixed with polymer(s) using a mortar and pestle for 10 min. Magnesium stearate and talc were added to the

above blend as flow promoters. In all the formulations the amount of Azathioprine was kept constant at 250 mg and the drug and polymers were taken in 1:0.72, 1:0.52, 1:0.32 (w/w) named G1- G3 for gaur gum and X1- X6 for xanthan gum polymer). The formulae of different matrix tablets of Azathioprine are given in the Table 2.

S. No.	Ingredients	Quantity (mg) Batch					
		G1	G2	G3	X4	X5	X6
1	Azathioprine	250	250	250	250	250	250
2	Guar gum	180	130	80	-	-	-
3	Xanthan gum	-	-	-	180	130	80
4	MCC	65	115	165	65	115	165
5	Magnesium stearate	5	5	6	5	5	5
		500	500	500	500	500	500

Table 2: Composition of Azathioprine matrix tablets

Evaluation of matrix tablets (8-17)

Physico-chemical characterization: The prepared matrix tablets were evaluated for weight variation, Uniformity of thickness, Friability, Hardness, stability study, FT-IR characterizations using reported methods.

Weight variation

Twenty tablets from each composition were weighed individually and average weight was calculated. Then the individual tablet weights were compared to the average tablet weight.

Thickness testing

The thickness of the matrix tablets was determined using screw gauge, and the results are expressed as mean values of 10 determinations.

Friability

Ten tablets were weighed and placed in the rotating disc of Roche friabilator. The apparatus was operated for four minutes at 25 rpm, dedusted and weighed again. The per cent of friability was calculated based on weight loss after the test.

Hardness

The tablet was placed between the two anvils of Monsanto hardness tester and increasing amount of force was applied. The reading was directly read on the marked scale till a pressure required to break tablet was recorded.

Drug content (11, 12)

For analysis of commercial formulation 20 tablets of Azathioprine were weighed and average weight equivalent to 10 mg taken in 100 ml volumetric flask and the volume was made up to the mark with methanol to give 100µg/ml concentration. From this 1 ml was taken and transferred to 10 ml volumetric flask and volume was made up to the mark with distilled water to give 10 µg/ml concentration. It was scanned on spectrophotometer in the UV range 200 - 400 nm. The spectrum was recorded at 280 nm. The concentrations of the drug were calculated from linear regression equation.

S. No.	Batch Code	% Wt Variation	Hardness (kg/cm^2)	Friability (%)	%Drug content
1	G1	3.960±0.35	5±0.05	0.2690.003	97.57±0.38
2	G2	1.996±0.27	5±0.10	0.335±0.005	98.78±0.21
3	G3	2.390±0.26	4.9±0.05	0.570±0.005	97.72±0.08
4	X4	1.988±0.17	5.2±0.05	0.701±0.05	97.27±0.15
5	X5	1.803±0.10	5.1±0.01	0.603±0.07	97.57±0.23
6	X6	3.792±0.33	5.1±0.05	0.403±0.070	99.48±0.38

Table 4: Evaluation of Formulated Tablets G1 to X6
All values are mean ±S.D for N=3

Compatibility study

The compatibility study was done by physical observation and by FT-IR. FT-IR spectra of azathioprine, guar gum and xanthan gum and azathioprine with guar gum and xanthan gum were recorded with FT-IR spectrophotometer (FTIR−8001, Shimadzu, Japan), operated with omnic software on sample prepared by KBR pellet method.
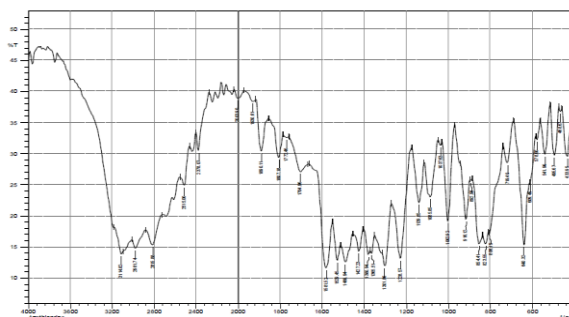

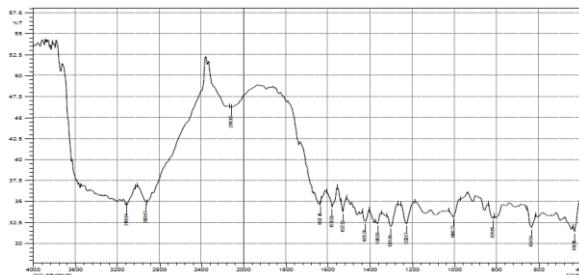**Figure 2 (A):** FTIR Spectra of Drug Sample (Azathioprine)
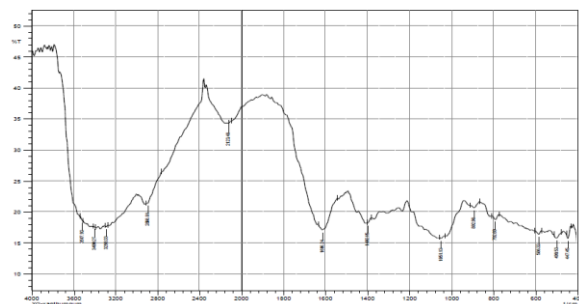
Figure 2 (C): FT-IR Spectrum of Xanthan gum

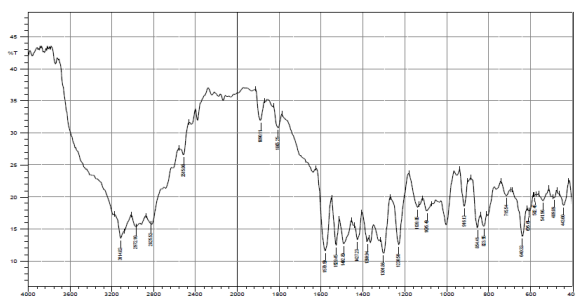**Figure 2 (C):** FT-IR Spectrum of Xanthan gum

Figure 2 (D): FT-IR Spectrum of physical mixture Guar Gum with Mixture
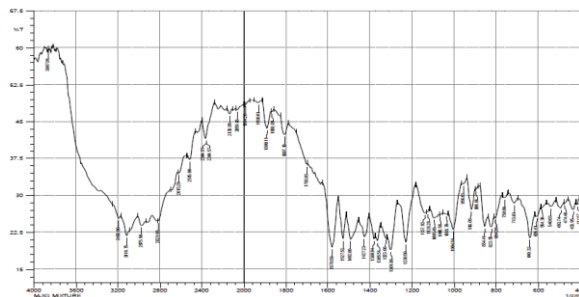

Figure 2 (E): FT-IR spectrum xanthan Gum with Mixture

Characteristics functional groups of drug (azatioprine)	Characteristic absorption	Mixture of drug with guar gum peak obtained	Mixture of drug with xanthan gum peak obtained
916 & 854.41	C-H Deformation	916.12	916.05
823.55 & 340.32	C-H Deformation	823.55	823.55 & 354
1228.57	C-N Stretching	1230.57	1230
1488.94 & 1380	C-H Bending	1492.80 & 1380.99	14892.80 & 1380.94
1529 & 1355.51	C-NO$_2$ Stretching	1529.59	1527 & 1365.51
1581.45 & 1529.45	C=N Stretching	1579.59	1579.45 & 1527.52
1880.11 &1807.8	C-H Deformation	1889.11 &1805.25	1890.18 &1807.8
2515	C-H stretching	2515	2515
2815	C-H stretching	2825.52	2821.66
2981	C-H stretching	2972.10	2975.96
3114	N-H Stretching	3114.82	3116.75
Guar gum			
3600-3300	OH stretching	3600-3300	3600-3300
3000-2900	Aliphatic CH stretching	3000-2900	3000-2900
Xanthan gum			
3600-3300	OH stretching	3600-3300	3600-3300
3000-2900	Aliphatic CH stretching	3000-2900	3000-2900

Table 3: FT-IR peaks and functional groups

Drug release studies in presence rat caecal contents

Due to similarity of human intestinal microflora with the rat caecal contents, the drug release studies were carried out in presence of rat caecal contents to assess the susceptibility of polysaccharides guar and xanthan gum to colonic bacteria. The rat caecal content 4% w/v was prepared as described in the previous section. The drug release studies were carried out using USP dissolution test apparatus I at 100 rpm and 37 $^\circ$C. The experiments were carried out initially in the same manner in 0.1M HCl and pH 6.8 phosphate buffer.

After this testing the dissolution medium was replaced with 500 ml beaker containing 200 ml of 4% w/v rat caecal contents in pH 7.4 PB which is kept in water batch of dissolution test apparatus. The experiment was carried with continuous CO_2 supply into beakers to simulate anaerobic environment of caecum. At different time intervals, 1 ml of the sample was withdrawn and replaced with 1 ml of fresh pH 7.4 phosphate buffer bubbled with CO_2 and the experiment or drug release studies were carried out for 24 hours since the usual colonic transit time is 20−30 hours. The volume of samples were finally made up to 10 ml with pH 7.4 PB and centrifuged. The supernatant was filtered through a bacteria proof filter and the filtrate was analyzed for azathioprine content at 280 nm. At the end of 24 hours, the tablet remnants were suspended in ethanol and the remaining drug content was estimated to make sure that the amount of drug remained, when added to the cumulative amount of the drug released up to 24 hours equals to the

average drug content of the tablets estimated prior to the drug release studies.

S. No.	Media	Time (h)	Mean per cent drug release					
			G1	G2	G3	X4	X5	X6
1		0.5	0.58±0.11	0.62±0.20	0.66±0.11	0.68±0.11	0.66±0.20	0.60±0.11
2		1.0	1.15±0.39	1.24±0.42	0.94±0.39	1.20±0.39	1.28±0.42	1.00±0.39
3	**0.1 N HCl**	1.5	1.26±0.39	2.59±0.50	2.64±0.39	1.29±0.39	2.64±0.50	1.24±0.39
4		2.0	2.08±0.19	3.28±0.45	3.34±0.19	2.13±0.19	3.34±0.45	2.04±0.19
5		2.5	2.49±0.03	4.36±0.44	4.44±0.03	2.54±0.03	4.44±0.44	2.48±0.03
6		3.0	3.18±0.45	5.42±0.59	5.53±0.45	3.32±0.45	5.53±0.59	3.13±0.45
7	pH 6.8 Phosphate buffer	3.5	3.50±0.45	6.92±0.50	6.98±0.45	3.62±0.45	6.98±0.50	3.48±0.45
8		4.0	3.94±0.50	7.08±0.50	7.18±0.50	4.02±0.50	7.35±0.50	3.91±0.50
9		4.5	4.29±0.50	8.29±0.49	8.33±0.50	4.49±0.50	8.50±0.49	4.26±0.50
10		5.0	4.63±029	9.32 ±0.44	9.39±0.29	4.89±029	9.710.44	4.59±029
Cumulative mean per cent drug release (Mean±SD; n=3)			**11.00%**	18.17%	17.54%	11.44%	19.25%	**10.82%**

Table 5: Drug Release Study of Formulation G1 to X6

S. No.	Media	Time (h)	Cumulative per cent drug released (Mean±SD; n=3)	
			G1	X6
1	7.4 Phosphate buffer with 0.4% RCC	8	40.02±0.44	47.12±1.07
		9	46.12±0.17	52.20±0.66
7		10	53.12±0.15	56.42±0.17
9		11	54.01±0.36	60.05±0.66
10		12	60.2±0.36	64.64±0.33
		24	70.01±0.17	79.06±0.50

Table 6: Cumulative Mean Percent Drug Released Study of Formulation G1 and X6 in Presence of RCC
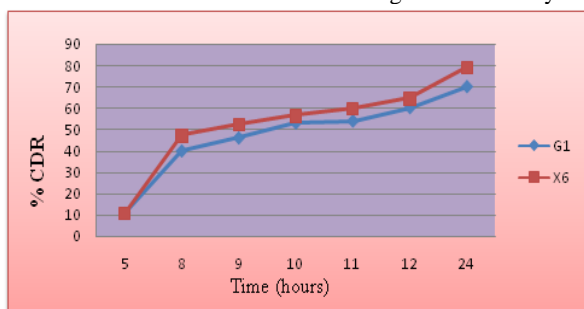

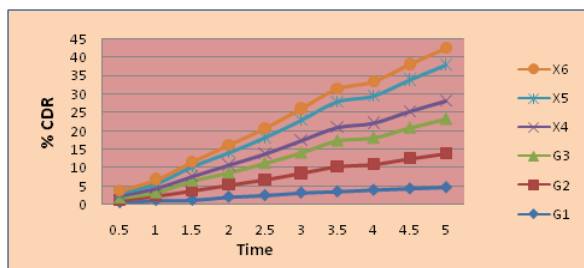**Figure 3:** Cumulative Percent Drug Released of G1, X6

Figure 4. Cumulative Percent Drug Release of G1, G2, G3, X4, X5, X6

Analysis of Swelling Index of Optimized Formulation

Measurement of the swelling Index was carried out to gain an insight into the phenomenon of polymer hydration and to evaluate the extent of media penetration within the tablets.

The swelling index was determined by equilibrium weight gain method. The study was carried out in the USP dissolution apparatus type 1. The tablets were accurately weighed, placed in dissolution basket, immersed in phosphate buffer (pH 6.8) and at regular intervals of 2, 4, 6, 8, 10 up to 24 h. The weighed basket matrix system was withdrawn from the dissolution vessel, lightly blotted with the tissue paper to remove excess test liquid and re-weighed. The swelling of each tablet was calculated according to the following equation and the results are recorded in table 7.

$$S.I. = (Wt-Wo)/W_O *100$$

Where Wt = initial weight , Wo = final weight

S. No.	Time Interval (h)	Swelling Index
1	0	0.00±0.00
2	2	52.00±0.25
3	4	90.07±0.56
4	6	133.55±0.25
5	8	160.17±0.23
6	10	188.88±1.15
7	12	191.85±0.89
8	24	45.02±0.75

Table 7: Swelling Index of Optimized Formulation (X6)

Stability Studies

The optimized formulation was subjected for two months stability study according to ICH guidelines**.** The selected formulations were packed in aluminium foil in tightly closed container. They were then stored at 40ºC / 75% RH for two months and evaluated for their permeation study.

S. No.	Model	R^2
1	Zero order	0.866
2	First order	0.958
3	Higuchi	0.920
4	Korsmeyer and peppas	0.962

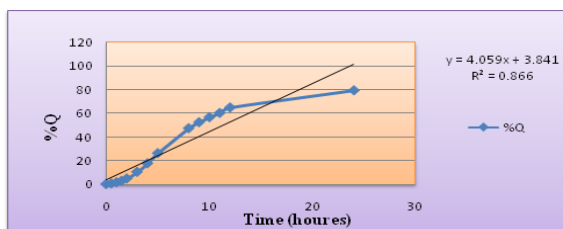**Table 9:** R^2 Value of Drug Release Kinetic Models

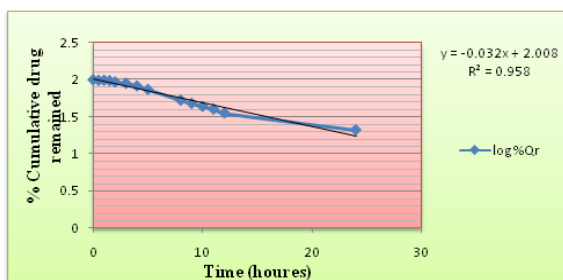**Figure 5:** Zero-order Model of Optimized Formulation X6

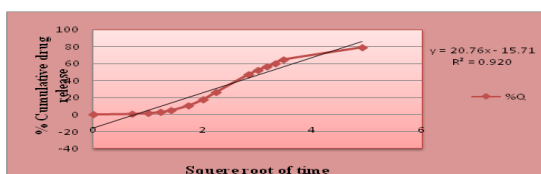**Figure 6:** First-order Model of Optimized Formulation X6

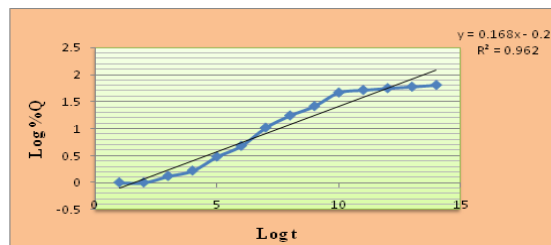**Figure 7:** Higuchi Model of Optimized Formulation X6

Figure 8: Korsmeyer and Peppas Model of Optitimized Formulation X6

Optimized formulation	Initial	After 30 days	After 60 days
X6	98.48±0.38	99.0±0.82	98.92

Table 10: Stability Study Data of Drug Content Uniformity of Optimized Formulation
Values are represented as mean ± SD (n=3)

Optimized formulation	Initial	After 30 days	After 60 days
X6	5.1±0.10	5.1±0.55	5.1±0.07

Table 11: Stability Study Data of Hardness of Optimized Formulation
Values are represented as mean ± SD (n=3)

Time (hrs.)	% cumulative drug release		
	Initial	After 30 days	After 60 days
0	0	0	0
0.5	0.58±0.35	0.57±0.32	0.56±0.63
1	1.56±0.56	1.52±0.77	1.51±0.45
1.5	2.78±0.54	2.75±0.34	2.71±0.71
2	4.8±0.85	4.8±0.54	4.8±0.32
2.5	7.26±0.68	7.24±0.12	7.22±0.33
3	10.36±0.78	10.30±0.55	10.26±0.19
3.5	13.74±0.49	12.74±0.93	12.70±0.52
4	17.54±1.22	17.50±0.13	17.00±0.70
4.5	21.69±0.85	21.40±0.12	21.20±0.81
5	26.13±0.73	25.99±0.51	25.99±0.11
8	47.12±1.07	46.12±1.06	46.10±1.02
9	52.20±0.66	51.20±0.42	51.20±0.16
10	56.42±0.17	55.42±0.07	55.22±0.01
11	60.05±0.66	59.05±0.33	59.03±0.11
12	64.64±0.33	63.64±0.22	62.64±0.11
24	79.06±0.50	79.06±0.50	78.06±0.50

Table 12: Stability Study Data of Dissolution Profile of Optimized Formulation
(Stability study was conducted at 40 ± 2^0 c/75±5% RH)

RESULT AND DISCUSSION:

The aim of any drug-delivery system is to provide a therapeutic amount of drug to the proper site in the body and maintain the desired drug concentration i.e. the drug delivery system should deliver drug at a specific rate.

Controlled drug delivery systems have been developed which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and/or targeting the delivery of drug to specific site.

In present scenario, sustained release matrix tablet were prepared by direct compression method. Optimization was done by using two polymers Guar gum, Xanthan gum.The enzyme galactomannase induced in rat caecal fluid hydrolyze polymeric linkages in guar and xanthan gum by the study of assessment of biodegradability of polysaccharides. As shown in table 1, the degradation of polysaccharides is more in presence of 4% w/v rat caecal content (RCC) in presence of galactomannase enzyme as compared to 2% rat caecal content as evidenced by decreased viscosity in 4% w/v rat caecal content than 2% rat caecal content. The control samples have not shown any drastic changes in the viscosity values. Due to this result 4% w/v rat caecal content was selected for the drug release with pH 7.4 phosphate buffer.

The compatibility study was done by physical observation and observing the results of drug-excipients compatibility study (physical), it was concluded that there was no incompatibility between drug and selected excipients. Hence, the excipients selected can be used with drug. The FT-IR spectra of azathioprine, guar gum, xanthan gum, and guar gum and xanthan gum with azathioprine have been shown in figure 2(A,B,C,D,E) respectively. The FT-IR spectra of drug, guar gum and xanthan gum were compared with FT-IR spectra of granules. FT-IR peaks (in cm-1) from the above table it was concluded that there were no change in the peak shape and no shift of peaks. So the drug was compatible with the polymers guar and xanthan gum.

From the above optimization studies it was concluded that with increase in concentration of Guar gum, there was a decrease in cumulative % release of drug, up to 5 hrs. and in case of Xanthan gum cumulative % release decrease of up to 5 h as the concentration of polymer is as decreased.

Now further dissolution study was performed for all selected batches from guar gum, xanthan gum and combination i.e. G1, and X6 batches of tablets was carried out for 24 h using U.S.P. XXIV (type II) dissolution rate test apparatus using 900 ml of 7.4 phosphate buffers in presence of RCC. The samples were analyzed for drug release by measuring absorbance using UV spectrophotometer.

X6 was selected as optimized formulation which shows 79.06% release of drug up to 24 h. The physical parameters of tablet X6 formulation i.e. weight variation; hardness, friability, and drug content were found to be 3.792±0.33%, 5.1±0.05 kg/cm2, 0.403±0.070, 99.48±0.38 respectively.

The mathematical models were used to evaluate the kinetic and mechanism of drug release from the tablets. The model that best fits the release data was selected based on the correlation coefficient (r) value in various models. The model that gives highest r value was considered as best fit of the release data.

Swelling of tablet involves the absorption of liquid resulting in an increase due to saturation of capillary spaces within the pa rticles or hydration pores and binds to large molecule, breaking the hydrogen bond a nd resulting in swelling of particles.

Measurement of the swelling Index was carried out to gain an insight into the phenomenon of polymer hydration and to evaluate the extent of media penetration within the tablets. Formulation X6 was showed 191.85±0.89% swelling Index and result concluded that formulation X6 showed good swelling Index.

By performing the release kinetic study, the drug release mechanism of matrix tablet (X6) was characterized. The release kinetic was showed that in-vitro release curve fitted under Korsmeyer and peppas model which show R^2 value 0.962 is highest as compared to other models. The regression coefficient R^2 value nearer to 1 indicated the model fitting that drug released from diffusion mechanism.

The best formulations X6 subjected to stability studies at 40 /75 RH and room temperature for 2 months. Then the tablets were analyzed for physical change, drug content and dissolution release estimation at an interval of 30 days. The study reveals the fact that visually no color change and minute changes in dissolution drug release as compared to initial was observed and there was slight degradation in drug content at the $25\pm2^{o}c/60\pm5\%$ RH. But there was a sudden fall in drug content at $40\pm2^{o}c/75\pm5\%$ RH, which showed that the drug should also be stored at a temperature $< 30^{0}c$ and away from light.

CONCLUSION:

In conclusion, the drug release retarding ability of various gums investigated was in the order XG>GG. Thus Xanthan gum has the ability to hydrate more rapidly than the guar gums used. The resulting drug diffusional path length for Xanthan gum was therefore the longest. Provided the gums have nearly similar diffusion coefficients, it would follow that the drug release rate from the Xanthan gum matrices would be

Sr. No.	Time t (hr)	CDR Q (mg)	$\sqrt{t}$	log t	%CDR %Q	log %Q	%Drug Remained %Q_r	log %Q_r
1	0	0	0	-	0	-	100	2
2	0.5	33.75	0.707	-	0.58	0.23	99.42	1.991
3	1	57.33	1.000	0.00	1.56	0.19	98.44	1.993
4	1.5	72.96	1.225	0.176	2.78	0.44	97.22	1.987
5	2	84.00	1.414	0.301	4.80	0.68	95.22	1.970
6	3	111.6	1.732	0.477	10.36	1.015	89.64	1.952
7	4	127.98	2.000	0.602	17.54	1.244	82.46	1.916
8	5	141.18	2.236	0.698	26.13	1.417	73.87	1.868
9	8	184.83	2.828	0.903	47.12	1.67	52.88	1.723
10	9	193.92	3.000	0.954	52.20	1.717	47.80	1.679
11	10	207.0	3.162	1.000	56.42	1.751	43.58	1.639
12	11	219.39	3.317	1.041	60.05	1.778	39.95	1.601
13	12	226.98	3.464	1.079	64.64	1.810	35.36	1.548
14	24	248.14	4.899	1.380	79.06	1.897	20.94	1.320

Table 8: Drug Release Kinetic Result (X6)

the slowest. The in vitro drug release studies revealed that, level of the polymer in the matrix tablets played an important role in the modulation of drug release.

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REFERENCES

1. Lee VHL. Changing needs in drug delivery in the era of peptide and protein drugs. In: Lee VHL. (Eds.). Peptide and protein drug delivery. Marcel Dekker Inc., New York,1-56.

2. Mishra P, Mahajan RP, Sharma A, Bhandari G, Mahajan SC. The Use of Azathioprine in Inflammatory Bowel Diseases- A Review. Int J Inv Pharm Sci 2013, 1(4); 300-314.

3. Srimornsak P, Nunthanid J, Wanchana S, Luangtana-Anan M. Pharm Dev Technol 2003, 8:311.

4. Krishnaiah YSR. J Control Release 1998, 55: 245.

5. El-massik MA, Nasra MA, Naggar VF. Development of metronidazole colon-specific delivery system. Asian journal of pharmaceutical sciences 2007, 2(1): 18-28.

6. Kumar V, Kumar S, mani Tamizh. Colon targeting drug delivery system: A review on recent approaches. Int J Pharm Biomed Sci 2011, 2(1):11-19.

7. Vandamme TH, Lenourry A, Charroeau C, Chaumeil JC. Carbohydr Polym 2002,48: 219.

8. Salve PS. Development and in vitro evaluation colon targeted drug delivery system using natural gums. Asian J Pharm Res 2011, 1(4): 91-100.

9. Asudani LH. M. Pharm Thesis-Formulation of solid dispersion of nimesulide with PEG 6000. University Department of Pharmaceutical Sciences. Nagpur 1999, 65-66.

10. Wells JI. in Pharmaceutical Preformulation: The physicochemical properties of drug. M. Pharm Thesis B. Ingoor1996.

11. Bhaskar M, Manohara YN, Gayasuddin M, Balaraju M and Bharath Kumar T. Spectrophotometric determination of azathioprine in bulk and pharmaceutical dosage forms. Int J of Chem Tech Research 2010, 2(1): 376-378.

12. Jain PS, Surana SJ. Development and validation of uv-spectrophotometric method for determination of azathioprine in bulk and in formulation. International journal of universal pharmacy and bio sciences 2012, 1(2): 49-58.

13. Lakade SH, Bhalekar MR. Formulation and evaluation of sustained release matrix tablet of anti-anginal drug, influence of combination of hydrophobic and hydrophilic matrix former. Research J Pharm and Tech 2008, 1(4): 410-413.

14. Pharmacopoeia of India, Vol. I, Ministry of health family welfare, Govt. of India, 1996:856.

15. ICH Q1A (R2) Stability testing guidelines: Stability Testing of new drug substances and products. [Online]. [Cited 2008 Nov 10]; Available from: URL:http://www.tga.health.gov.au/docs/ pdf/euguide/inch/273699r2en.pdf

16. Biopharmaceutics and Pharmacokinetics A Treatise. 1st ed. (D.M. Brahmankar, Sunil B. Jaiswal, ed.), Vallabh Prakashan, New Delhi, 1995: 224-225.

17. Modern Pharmacology, 4th ed. Charles R. Craig, Robert E. Stitzel, ed., Little, Brown and company, New York, 1994: 491.