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Development and Evaluation of Colon Specific Matrix Tablets of Oxaliplatin combined with Diclofenac sodium

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ABSTRACT

Colon specific tablets of Oxaliplatin combined with anti-inflammatory agents, Diclofenac sodium were prepared using guar gum as matrix carriers in varying concentrations from 40% to 65%. The drug combination in the tablet was estimate simultaneously using newly developed and validated UV derivative spectroscopy method. *In vitro* drug release profile was studied in changing media method, first in 0.1N HCl for two hours followed by 3 hours in phosphate buffer media, pH 7.4 (PB7.4) and in simulated colon fluid (phosphate buffer pH 6.8 added with rat ceacal content) (SCF) for 19 hours. The drug release profiles from PB7.4 and simulated colon fluid were found to be dependent on the gaur gum concentration. Matrix tablets of oxaliplatin and diclofenac sodium combination with 60% w/w Gaur gum showed a total release of ~66% of Oxaliplatin and ~53% of Diclofenac sodium after 24 hrs. The colon tissue homogenate studies conducted after oral administration of the optimized matrix tablet in New Zealand Rabbits showed 156 µg oxaliplatin and 96 µg Diclofenac sodium recovery in 24 hours. X-ray Images of matrix tablets containing barium sulphate in Rabbit showed tablets to be intact in small intestine (6 hours after administration) but were diffused and spread out in large intestine and colon confirming enzyme mediated erosion of the tablet in these regions.

Keywords- Matrix tablets, Guar gum, Oxaliplatin, Diclofenac sodium, Colorectal cancer, controlled release.

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INTRODUCTION

Colorectal cancer causes many deaths worldwide per year. Many colorectal cancers are thought to arise from adenomatous polyps in the colon. These mushroom-like growths are usually benign, but some may develop into cancer over time. The tumor typically begins as a noncancerous polyp, a growth of tissue that develops on the lining of the colon or rectum that can become cancerous[1] .There are many risk factor for colorectal cancer including Heredity and family history[2], Personal medical history[3], Diet[4], Alcohol and smoking[5], etc. The main types of treatment that can be used for colon and rectal cancer are Surgery, Radiation therapy, Chemotherapy, Targeted therapy. Depending on the stage of the cancer, two or more of above treatment can be combined at the same time or used one after the other[6]. Oxaliplatin is the third generation analogue of cisplatin that have activity on colon cancer. The most successful combination of oxaliplatin is with other drugs such as irinotecan (CPT11), for which response rates up to around 60% were reported (Carrato A et al)[7]. Many epidemiological studies have shown the efficacy of non steroidal anti inflammatory drugs (NSAIDs) in inhibiting a variety of cancers including colon, skin, bladder etc (Ruegg C. et al 2003)[8]. They act by inhibition of COX-2 enzyme which is seen to be upregulated in various malignancies (kaur J et al 2010)[9]. The effects of the chemopreventive agents in vitro have been widely used with growing evidence that it may provide antitumor efficacy, particularly when targeted against colorectal cancer, either alone or in Combination (Campbell FC et al 2005; Sharma RA et L 2005)[10,11] .

Targeted delivery of drugs to colon reduces not only the systemic side effects but also provide safe therapy for colon cancer. Among the various routes of targeting, oral route is the route of choice among the patients because of ease of administration. Targeting of drugs specifically to the colon is advantageous for the treatment of diseases associated with the colon such as amaebiasis, ulcerative colitis, and colorectal cancer (Jain A et al.2007)[12]. Guar gum is being used to deliver drug to colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine. Colonic bacteria are fundamentally *anaerobic* in nature and are involved in the fermentation of carbohydrates and proteins that have escaped digestion in the stomach and small intestine (Kinget R et al.1998)[13]. These anaerobic bacteria are responsible for the degradation of guar gum in the colon (Macfarlane G.T et al 1990)[14]. Colon specific drug delivery system for drugs like salicylic acid (Krishnaiah et al., 1999)[15], Dexamethasone using gaur gum is reported (Wong D. et al 1997)[16]. Gaur gum matrix tablet of Dexamethasone degraded completely in the colon and could release 72-82 % of the drug in the colon (Kenyon,

C.J et al 1997)[17]. Combination of guar gum with other polymers is also studied for colonic delivery. Matrix tablet of chitosan and guar gum of Diltiazem hydrochloride coated with inulin followed by shellac showed that the tablets coated with inulin and shellac have controlled the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment (Valluru, R.et al 2007)[18].

The objective of the present study was to develop and to evaluate guar gum matrix tablet for colon specific delivery of Oxaliplatin (OXP) with Diclofenac sodium (DFS). A simple and precise simultaneous analytical method was developed to estimate both the drugs in combination in tablet, dissolution media and in colon tissue homogenate using derivative spectroscopy. Optimized matrix tablets were evaluated for colon delivery in-vivo in normal rabbit animal model. Intactness of the tablets during intestinal transit was tested in rabbits by x-ray imaging.

MATERIALS AND METHODS

Materials

The drug sample Oxaliplatin was obtained from Panacea Biotech, Mohali, Punjab (India). Diclofenac sodium and gaur gum were obtained from Hi media laboratories Pvt. Ltd. Mumbai (India). Talc, Magnesium stearate, starch and Microcrystalline cellulose was obtained from CDH Analytical Reagents New Delhi. All other chemicals are of laboratory grade and were procured from local suppliers.

Simultaneous estimation of OXP and DFS in combinations:

Preparation of stock and standard solutions:

Weighed accurately 20 mgs of OXP and was dissolved in 20ml of 0.1N HCl to produce a stock solution of 1000 µg/ml. Stock solution was suitably diluted with 0.1N HCl to prepare aliquots of test solution in the concentration range of 20 to 200µg/ml. Similarly, stock solutions and test solutions of OXP in phosphate buffer (pH 7.4) and phosphate buffer saline (PBS) (pH 6.8) were also prepared.

Weighed accurately 5 mgs of DFS and was dissolved in 50ml of methanol to produce a stock solution of 100 µg/ml. Stock solution was suitably diluted with 0.1N HCl to prepare aliquots of test solution in the concentration range of 2 to 20 µg/ml. Similarly, stock solutions and test solutions of DFS in phosphate buffer (pH 7.4) and phosphate buffer saline (PBS) (pH 6.8).

Stock solutions containing OXP-DFS combinations were prepared by mixing 50 ml of individual stock solutions of OXP and DFS. Test solutions were prepared by dilution with the respective solvents as indicated above.

Simultaneous estimation of drugs in combinations:

The zero-order absorption spectra of OXP-DFS test solutions were taken using UV/Vis spectrophotometer solutions in the wave length range between 200-400 nm. Since the spectra display overlapping in the region it was difficult to determine OXP in the presence of DFS. Hence, the zero order spectra were converted to first order derivative spectra using delta lambda-4 software at scaling factor of 10, to find out the zero crossing wave lengths of OXP and DFS in 0.1 N HCl (Figure 1a), PBS, pH 7.2 (Figure 1b) and PBS, pH 6.8 in presence of cecal content (Figure 1c). The zero cross wave lengths observed were different with different media and are tabulated in Table 1.

Accordingly, first derivative spectra of different concentrations of OXP + DFS were taken for the generation of calibration curve in all the three media (Figure 2a, 2b, 2c). Regression analysis was carried out for the data obtained to determine slope, intercept and correlation coefficient.

Analytical method validation:

Validation of the analytical method for the simultaneous estimation of OXP with DFS was conducted. The parameters of linearity, accuracy, inter-day precision, intra-day precision, limit of detection (LOD) and limit of quantification (LOQ) were evaluated to validate the process. Linearity was established by regressing the analytical data to obtain regression equation and correlation co-efficient (r^2). Accuracy of the method was determined by the recovery studies conducted with the prepared tablet formulations containing OXP with DFS by the addition of known quantities of standard drug solution to pre-analyzed samples. Experiments were repeated three times in a day to determine intra-day precision and on three different days to establish inter-day precision. The relative standard deviation (RSD) was calculated in each analysis. LOD and LOQ were calculated by repeating the blank measurements six times at first order λmax determined previously for OXP with DFS.

Method of preparation of Matrix Tablets:

Matrix tablet is prepared by wet granulation technique. MCC was used as diluent and Magnesium stearate and Talc were used as Lubricant (Munira Momin et al 2004)[19]. Guar Gum was included in Formulations in various proportions (40 to 65%) to prepare matrix tablet

Firstly, the Guar gum was sieved separately and mixed with drug and MCC. Then the Powder is blended and granulated with 10 % (w/v) starch paste. The Wet Mass obtained was passed through a mesh (#16). Then The granules obtained after passing the wet mass through the sieve was dried at 60^{o}C. The Dried granules were again passed through a mesh (#22). Lubrication was

done with a mixture of Talc and Magnesium Stearate. At the end Granules were compressed with 8mm punch.

EVALUATION OF MATRIX TABLET

All the Guar gum matrix tablet formulations were evaluated for Weight variation, Hardness and Friability as per pharmacopoeial methods [20]

Drug content uniformity- Five matrix tablets were weighed and powdered quantitatively and mixed in 50ml methanol. The mixture was shaken well and added sufficient methanol to produce 100 ml, mixed well and filtered. Diluted 10 ml of the above solution to 100 ml with methanol. The drug content was estimated by the method described above.

In-vitro dissolution studies

In–vitro dissolution studies of matrix tablet were conducted in USP II Dissolution apparatus (paddle type) at 100rpm,in 900 ml of 0.1 N HCL for 2h (average gastric emptying time) .Then the medium was replaced with Phosphate buffer (pH 7.4) for 3h (average small intestine transit time). Then the medium was replaced by Phosphate Buffer (pH 6.8) for 19hrs in one set and in the other, the medium was replaced by Phosphate Buffer (pH 6.8) contain 2% w/v rat cecal content. At specified time intervals (2h, 5h, 8h, 12h, 16h, 20h, 25h) 5ml of sample is withdrawn and replaced with 5 ml of respective fresh buffer. The withdrawn sample was analyzed for percent drug content using UV Derivative Spectroscopy method developed. Mean results of triplicate measurements and standard deviation were reported.

For rat ceacal content the abdomen of rats were opened, the cecum was traced, ligated at the both ends, dissected and immediately transferred into PB(pH 6.8) previously bubbled with carbon dioxide. This was finally added to dissolution media and study was continued.[19]

Colon tissue homogenate studies

The tissue homogenate studies of the optimized Guar gum matrix tablets (GG/OD-60) were conducted in New Zealand Rabbits (2 to 3kg). Animals were housed with free access to water and food. The study protocol as approved by Institutional Animal Ethical committee of I.S.F College of pharmacy was followed. The studies were carried out as per the guidelines of council for the purpose of control and supervision of experiment on animals (CPCSEA),Ministry of social justice and environment, Govt. of India.

Matrix tablet was administered to Rabbits by oral route. After 12hr the rabbits were sacrificed and colon was isolated, homogenized and the homogenate was subjected to extraction and was estimated for the percentage of drugs by UV derivative spectroscopy method as described below.

Estimation of drugs in colon tissue homogenates:

Estimation of Oxaliplatin and Diclofenac sodium in colon tissue, were conducted by the first derivative spectroscopic method described above. Rabbit was sacrificed by cervical dislocation and the colon was removed. The colon was washed with phosphate buffer and colon sample was homogenized in Phosphate buffer. One portion was kept aside which served as blank and the other portion was divided in to required number of portions and added with known quantities of all the drug solution separately and stirred well. These suspensions were centrifuged (8000-10000 rpm) for 20 minutes at an ambient temperature. After centrifugation, supernatant was transferred into clean, fresh volumetric flask; volume made up to mark and was estimated by the UV Derivative Spectroscopy method respectively developed for all the drugs. Calibration curve was plotted for the estimation of OXP (Figure 3a) and DFS (Figure 3b).

Radiographic monitoring of orally administered tablet:

The radio-opaque tablets were prepared using barium sulphate as per the optimized formulation just by replacing Drugs with sufficient quantity (20 mg) of barium sulfate and diluents. The other parameters of tablet formulation were kept constant. The *in-vivo* GIT study was carried out by administering a Barium sulphate tablet using a Intravenous feed tube to the overnight fasted New Zealand Rabbits (2.5-3.0 kg) and monitoring them through radiological method.

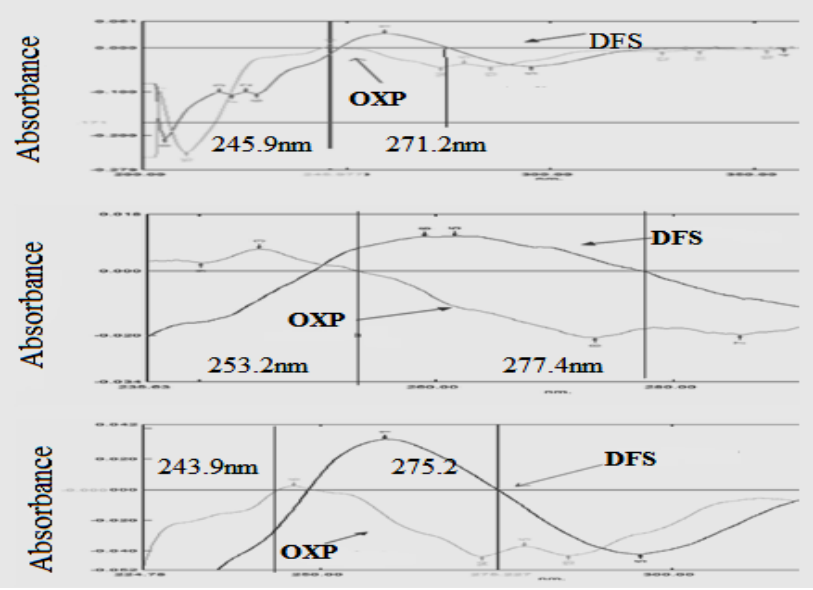

Figure 1: Zero cross over spectra of OXP and DFS combination in (a) 0.1 N HCl (Spectra of OXP crossing zero line at 245.977 nm and DFS at 271.2 nm) (b) PBS pH 7.2 (Spectra of OXP crossing zero line at 253.2 nm and DFS at 277.4nm) and (c) PBS pH 6.8 with cecal content (Spectra of OXP crossing zero line at 243.9 nm and DFS at 275.2nm) where DFS and OXP can be estimated in the respective media with out interference of the other drug.

RESULT AND DISCUSSION

Simultaneous estimation of OXP and DFS in combinations:

First derivative spectroscopic method for the simultaneous estimation of OXP in presence of DFS and DFS in presence of OXP. Zero order spectra of the drug combination showed interference with each other and hence first order derivative spectra (Figure 1) were developed in different media. Zero crossing wave lengths were identified and recorded (Table 1). Calibration curves generated for OXP and DFS estimation in their combination showed linearity with coefficient of determination (r^2) near unity in the concentration range between 50 to 200 µg/ml for OXP and 5 to 20 µg/ml for DFS. Regression parameters are presented in table 1.

Table1. Summary of validation Parameters

Parameters	**OXP:DFS Combination**					
	0.1N HCl		**PBS (pH 7.2)**		**PBS (6.8) With Cecal content**	
	OXP	**DFS**	**OXP**	**DFS**	**OXP**	**DFS**
Zero Crossing λ(nm)	271.2	245.9	277.4	253.2	275.2	243.9
Linearity Range(µg/ml)	50-200	5-20	50-200	5-20	50-200	5-20
Slope (Mean)	0.0092	0.0082	0.00111	0.00595	0.00111	0.00111
Intercept	+0.00047	+0.003	-0.007	-0.005	-0.005	0.000
R^2	0.9982	0.996	0.997	0.996	0.995	0.998
Accuracy as	97.215±	97.63±	96.2±	96.96±	97.33±	95.15±
% Recovery	2.349	2.138	0.14	0.05	0.57	0.25
Std Dev.(SD)	0.00047	0.000341	0.00017	0.000243	0.000192	0.00019
RSD	0.0511	0.0416	0.1504	0.04084	0.1734	0.1734
LOD	0.1686	0.1372	0.4965	0.1348	0.5724	0.5724
LOQ	0.511	0.4159	1.504	0.4084	1.734	1.734
%RSD (Intra day)	0.944	0.934	0.968	0.916	0.912	0.935
% RSD(Inter day)	1.344	1.112	1.246	1.513	1.154	1.554

Validation:

Validation parameters determined under different media are tabulated in table 1. The intra-day and inter-day precision of the method was evaluated by means of six determinations at 100% of their respective test concentration. In the analysis of the OXP with DFS the RSD values for intra-day (n=6) and inter-day (n=6) precision were less than 2% indicating the precision of the method for estimation in combination. Accuracy was evaluated by the standard addition method. The mean percentage recovery obtained was above 95% for both OXP and DFS. These recovery values indicate the accuracy of the method developed. Linear regression analysis of the analytical data showed low intercept values (Between 0.003 to 0.0047). These results indicate no interference of DFS in the estimation of OXP and interference of OXP in DFS estimation.

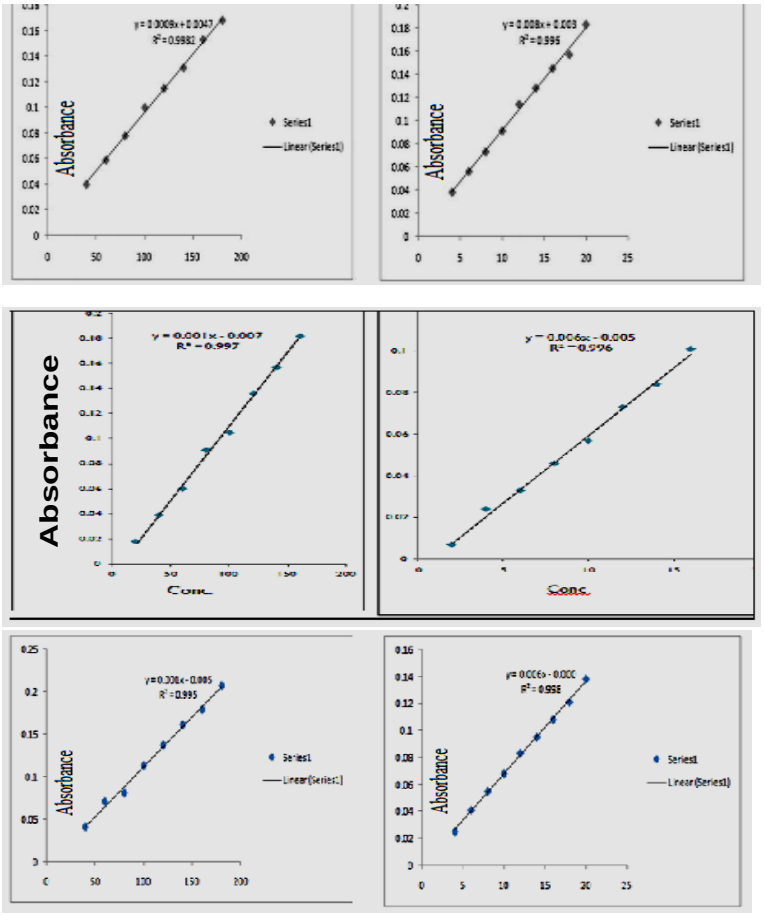

Figure 2: Calibration curve of OXP (Left) and DFS (Right) for their simultaneous estimation by first derivative spectroscopy in (a) 0.1 N HCl, (b) PBS pH 7.2 and (c) PBS pH 6.8 with cecal content.

Evaluation of Matrix Tablets

Matrix tablets are an interesting option when developing an oral controlled release formulation. Different batches of matrix tablets prepared are listed in table 2. The concentration of drugs, Starch, Magnesium stearate, Talc was kept constant in all formulations. The concentration of guar gum (40 to 65%) and diluent (MCC) were varied and accordingly six formulations were prepared. All tablets were prepared by wet granulation method.

Table 2: List of Tablet formulation

Ingredients (per Tablet)mg	GG/OD-40*	GG/OD-45*	GG/OD-50*	GG/OD-55*	GG/OD-60*	GG/OD-65*
Oxaliplatin	20	20	20	20	20	20
Diclofenac Na	16	16	16	16	16	16
Guar gum	80	90	100	110	120	130
MCC	58	48	38	28	18	8
Starch	20	20	20	20	20	20

***Indicate gaur gum 40%, similarly other batches.**

The results of matrix tablet evaluation parameters are listed in table 3. Weight variation (2.56 to 4.83 %) percentage Friability (< 1%) and drug content (>90%) were in compliance with the standard limits. Tablets showed hardness in the range between 4.53 to5.33 kg/cm^2. being a matrix tablet Hardness was kept high enough (4.53 to5.33 kg/cm^2) to avoid disintegration before reaching colon. Percentage drug content determined was above 90 % for all the samples.

Table 3: Evaluation of various parameters

Formulation	Hardness (kg/cm^2)	Weight Variation %	%Friability	Drug content% (Oxaliplatin)	(Diclofenac)
GG/OD-40	4.53±0.05	2.56±0.11	0.25±0.01	93.98±0.14	96.16±0.28
GG/OD-45	4.7±0.05	4.63±0.05	0.39±0.005	94.33±0.08	90.96±0.05
GG/OD-50	4.83±0.05	3.56±0.05	0.43±0.005	96.2±0.14	92.23±0.40
GG/OD-55	5.06±0.05	5.4±0.1	0.65±0.005	95.33±0.57	95.96±0.05
GG/OD-60	5.26±0.05	3.43±0.05	0.55±0.005	92.66±0.28	93.15±0.25
GG/OD-65	5.33±0.05	4.83±0.05	0.50±0.005	97.33±0.28	92.06±0.11

In-vitro drug release studies:

In- vitro dissolution of all formulations was carried out in different medium; 0.1 N HCL for 2hr, Phosphate Buffer (pH 7.4) for 3hr and Phosphate Buffer (pH 6.8) for 19 hr with or without Rat cecal content. Concentration of gaur gum used was between 40% to 65% at 6 levels. Selection of gaur gum percentage was made based on the in-vitro drug release data looking in to the following criteria;

1. Tablet integrity throughout the GI transit up to colon
2. Minimum drug release in stomach and small intestine (First 5 hours)
3. Maximum drug release in colon

GG/OD-40 and GG/OD-45 matrix tablets disintegrated within 6 hours and so were rejected and the remaining tablets (GG/OD-50, GG/OD-55, GG/OD-60 and GG/OD-65) were subjected to in-vitro release studies in changing media with and without cecal content. Release profile of oxaliplatin from GG/OD matrix tablets in to the combined media without cecal content and with ceacal content are shown in figure 4. Tablets containing lower Gaur gum content (GG/OD-50 and GG/OD-55) showed high release in PBS pH 7.2 (Within 5 hours after administration) and hence were not selected for the detailed study.

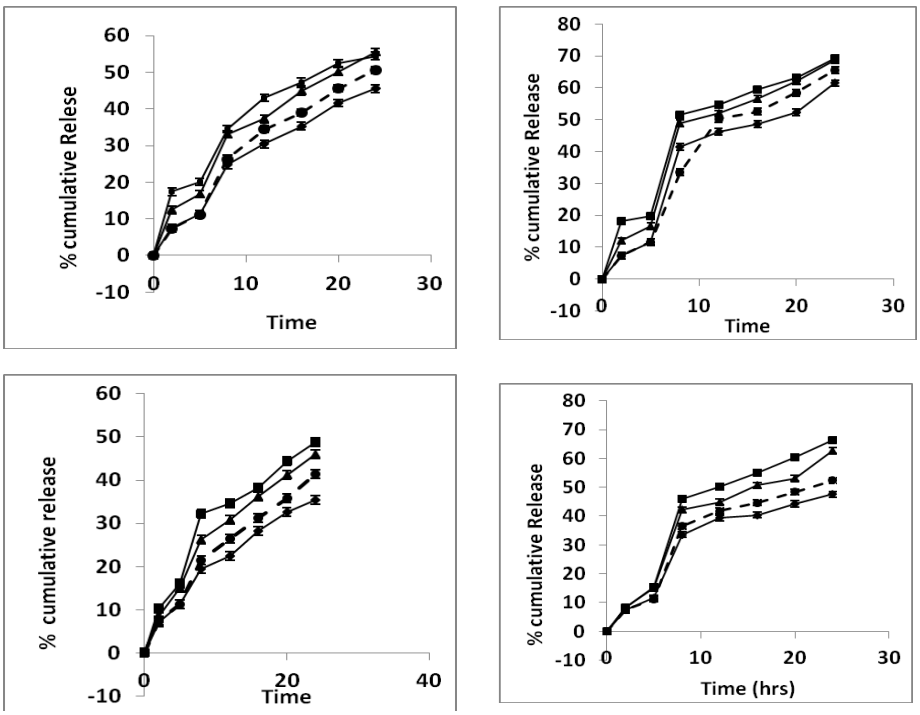

Figure 3: *In –vitro* Release of OXP (Upper) and DFS (Lower) from GG/OD-50(■), GG/OD-55(▲), GG/OD-60(●) and GG/OD-65(♦) matrix tablets in to changing pH media without caecal content (Left) and with caecal content (Right).

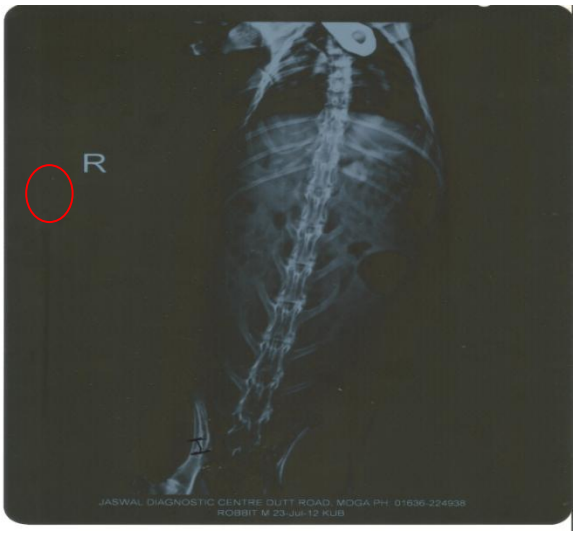

Figure 4: X-ray Images of Rabbit were taken 3hr after oral administration of Barium sulphate containing gaur gum matrix tablet. Image above shows the intact tablet (circle) in the region of small intestine in the lower abdominal cavity.

In-vitro drug release from matrix tablets:

Drug release studies from GG/OD-60 and GG/OD-65 showed convincing release behavior (~10%) in first 5 hours. Total drug release in the last Phase of 19 hours of release study in media with caecal content was high from GG/OD-60 than GG/OD-65 obviously due to higher

resistance for break down and drug release from GG/OD-65 tablets. Hence GG/OD-60 was selected for in-vivo studies. Total drug release from GG-60 tablets into medium without ceacal content was 50.58% (Oxaliplatin) and 41.32% (Diclofenac sodium) at the end of 24 hrs while with caecal content medium, the release was 65.55% of Oxaliplatin and 52.42% of Diclofenac sodium in 24 hours. In other words release studies carried out in presence of rat cecal content showed 10% to15% more drug release. This indicates the importance of the intestinal bacteria and the enzyme they secrete in the degradation of polysaccharide like gaur gum and drug release specifically in colon. In comparison, percentage release of oxaliplatin was higher than that of diclofenac sodium in all tablets. This may be due to low solubility of DFS in comparison to OXP in most of the regions of GI tract.

Colon tissue homogenate studies

The objectives of the study being the maximum delivery of both the drugs in to colon after oral administration, it was rational to estimate the drug content in colon 12 hours after administration. Since small amount of absorption do take place in colon and appreciable amount of the drug is retained in colon tissue, it was thought to excise the colon tissue and estimate the drug content 12 hours after oral administration. Tissue samples homogenates were extracted and estimated for both the drugs. Percentage of the total drug recovered in colon was also calculated.

Concentration of oxaliplatin and diclofenac was estimated by first order derivative spectrometric method at the absorption maxima 278.9 nm (Oxaliplatin) and 251.1nm (Diclofenac sodium). The concentration of Oxaliplatin recovered in the colon tissue after 12 hrs after the administration of GG/OD-60 matrix tablet was 156 µg/ml and concentration of Diclofenac sodium was 96 µg/ml.

X-Ray imaging studies:

X-ray Images of Rabbit were taken 5 hr after oral administration of Barium sulphate containing gaur gum matrix tablet. Images obtained showed that the tablet observed were intact in the region recognized (circled) at the lower abdominal cavity probably in the small intestine.

CONCLUSION:

From these studies it was concluded that the 60% guar gum matrix tablet gives significantly less drug release in first 5hr (stomach and small intestine transit time) but the release in colon was significant than the other formulations tested. The release of oxaliplatin in colon is to the order of 65 to 70% of the total amount in the tablets. Diclofenac release from GG/OD tablets was significant (55 to 60%) but less than that of oxaliplatin. Further studies are required to increase the availability of both the drugs in colon to exploit the observations made In this study in

potentiating the action of oxaliplatin when combined with NSAIDs drugs like DFS. This study also warrants for a detailed study for effectively using this combination to reduce the dose of Oxaliplatin not only to potentiate the activity but also avoids adverse effects of Oxaliplatin by dose reduction in colorectal cancer.

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