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Development And Evaluation of Colon Specific Drug Delivery System Via pH and Microbial Triggered Mechanism for Colon Cancer

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ABSTRACT

The assumption on colon-specific drug delivery system suffers from minor inherent problems. The development of novel into pH dependent and microbially triggered compression enteric coated tablets was done. Capecitabine was used as a model drug. The core tablet was coated with acid soluble coating, Eudragit®S-100 which was compression coated with immediate release blend of drug containing microbial triggering polysaccharide, guar gum. Different combinations of polymers were selected to achieve drug targeting to the colon for the treatment of colorectal cancer. The novel – CODES successfully showed resistance to the gastric environment and exhibited no drug release in simulated intestinal fluid. In-vitro release studies for prepared tablets were carried out for 2 hours in 0.1 N HCl, 3 hours in pH 7.4 phosphate buffer and remaining in 6.8 pH phosphate buffer. In vitro studies revealed that it have limited drug release in stomach, small intestine and released maximum drug in the colonic environment.

Keywords: pH dependent, time delayed, microbially triggered, compression coating, CODES^{TM}.

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INTRODUCTION

Cancer that begins in the colon is called colon cancer, while cancer that begins in the rectum (the last six inches of the rectum) is known as rectal cancer. Cancers affecting either of these organs also are referred to as colorectal cancer.[1] Colorectal cancer is the second leading cause of cancer – related deaths in Europe and the USA. It ranks second in incidence to lung cancer in men and breast cancer in women. More than 900000 new cases of colorectal cancer are diagnosed each year and colorectal cancer accounts for nearly 500000 cancer deaths worldwide annually. There is no difference in incidence between men and women and this kind of cancer is prevalent in individuals aged over fifty. Colon cancer often results from a combination of genetic predisposition and environmental factors. Such factors include a diet low in fiber, vegetables and folate and high in fat, red meat; heavy alcohol consumption; a sedentary occupation; and cigarette smoking. Although colorectal cancers may appear at different times and for different reasons, they share a common random pathway from normal epithelium through polyp to carcinoma.[2]

Colon specific drug delivery system is considered to be beneficial in the treatment of colon diseases. The colon is a site where both local and systemic drug delivery could be achieved, topical treatment of inflammatory bowel disease, for example Ulcerative Colitis or Crohn's disease. Such inflammatory conditions are usually treated with glucocorticoids and sulphasalazine (targeted). A number of others serious diseases of the colon, e.g. colorectal cancer might also be capable of being treated more effectively, if drugs were targeted to the colon. Formulations for colonic delivery are also suitable for delivery of drugs which are polar and/or susceptible to chemical and enzymatic degradation in the upper gastrointestinal tract highly affected by hepatic metabolism, in particular therapeutic proteins and peptides.[3]

Colorectal cancer is classified into four distinct stages along with a fifth stage called "recurring". According to American Joint Committee on Cancer, each stage has different treatment options with a five-year survival rates.

Stage 0:

It is the very early stage of colon cancer where polyps are formed in the mucosal lining of the colon. During colonoscopy, the polyps are eradicated fully by polypectomy. This prevents the advanced stages of colon cancer to occur.

Stage I:

At this stage polyp develops into a tumor and invades the inner-lining of the mucosa. Usually surgery is the main option for treating the colon cancer at this stage where the cancerous portion of

the tissues is separated from the non-cancerous portion. Survival rate is around 95% if colon cancer is detected at this stage.

Stage II:

It is characterized by whether the cancer has spread beyond colon but not to the lymph nodes through metastasis. This stage is subcategorized into Stage IIA, Stage IIB and Stage IIC depending on the spreading of cancer to the muscular layer or outermost layer of the colon or beyond colon. Resection surgery is the only option to treat this stage of colon cancer and the survival chances of the patients at this stage is 85%.

Stage III:

This stage of colon cancer is diagnosed with cancer has already spread all the wall of the colon and also to the surrounding lymph nodes and the survival rate is around 30–60%. This stage of cancer is subcategorized into stage IIIa, b and c depending on the spreading of the cancer to the inner, middle and outer layer of colon and the surrounding lymph nodes. Along with the surgery, chemotherapy and the other medical therapy is required to treat this cancer.

Stage IV: This stage the cancer has speeded to the other part/organ of the body like liver, ovary, testis, intestines. Survival rate is only 3%. Surgical resection, chemotherapy, radiation therapy and surgical removal of the portion of the other body parts with cancer are operated to treat at this stage of colon cancer.[4]

Treatment depends on general health as well as the type, stage and grade of the cancer. For colorectal cancer, treatment may include a combination of surgery, radiation therapy, chemotherapy and biologicaltherapy.[5] Surgery is the main treatment for colorectal cancer. Surgery is used to cure the cancer in the early stages by completely removing the tumor and tissues affected by it.[6,7] Radiation therapy destroys cancer cells, which grow uncontrollably, but it also can damage healthy cells near by.[8]

Colon specific drug delivery system has attracted considerable attention for the past few years in order to develop drug delivery systems that are able to release drugs specifically in the colon in a predictable and reproducible manner. The colon is a site where both local and systemic delivery of drugs can take place. To achieve successful colon targeted drug delivery, a drug need to be protected from degradation, release and absorption in the upper portion of the gastrointestinal tract (GIT) and then to be ensured abrupt or controlled release in the proximal colon.[6]

MATERIALSAND METHOD

Materials

Capecitabine was purchased from Swapnroop drug and Pharmaceuticals, Aurangabad, India. EUDRAGIT (S-100) and EUDRAGIT (L-100) polymers was supplied by Evonik Parma Mumbai, India. Other excipients used to prepare the core tablets such as Lactose, Talc, Magnesium stearate and micro crystalline cellulose (MCC) was supplied by Loba chemicals Pvt. Ltd. Mumbai, India were of analytical grade.

Method of preparation

The MCC binder was dissolved in water to get a clear solution. Then in another beaker Capecitabine, polymers were mixed for 15mins. This Mixture were sifted through #40 sieves. The binder solution mixed with Capecitabine, polymers mixture to get wet mass form and that wet mass was passed through No.18 sieve. Wet granules formed and were dried in a Rapid drier at 50^{o}C for 60 minutes. The dried granules were sifted through # 20sieve.The fines were separated using 40# sieve to 20-40# granules. These granules were lubricated with magnesium stearate and glidant talc are mixed. The granules were compressed into tablet using mini press tablet compression machine. Composition of core tablet of Capecitabine was shown in Table 1.

Table 1: Composition of core tablet of Capecitabine

Sr.No	Ingredient	Quantity (mg)	Category
1	Capecitabine	150	Anti-Cancer
2	Sodium starch glycolate	5	Super disintegrating
3	Lactose	24	Diluents
4	Micro crystalline cellulose	7	Binder
5	Magnesium stearate	2	Lubricant
6	Talc	2	Glidant
7	Water	Q.S	Solvent

Drug Excipients Compatibility Study

Drug Excipients Compatibility Study was carried out by Fourier Transform infrared spectroscopy (FT-IR spectrophotometer, Agilent Technologies, carry 630 FTIR.). The sample was analyzed in the region of 4000 and 400cm^{-1}. The mixture of drug and polymer in 1:1 ratio were mixed well and analyzed for FTIR spectroscopy.

Differential Scanning Calorimetry (DSC)

Thermograms were obtained by using a differential scanning calorimeter at a heating rate 10^{0} C/min over a temperature range of 0-300^{0}C. The sample was hermetically sealed in an Aluminium crucible. Nitrogen gas was purged at the rate of 10 ml/min for maintaining inert atmospheres.

Bulk Density, Tap Density, Carr's Index and Hausner's Ratio:

Bulk Density and Tap Density were subjected to Bulk and Tap density determination. Tapping cylinder method was used for determining bulk and tap density of granules were taken in 10 ml measuring cylinder. Initial volume (Bulk volume) and the volume after 100 tapings (Tap volume) were measured. From the results of Bulk and Tap densities, the Hausner's ratio and Carr's Index were calculated. Each micrometric property was performed in triplicate manner and reported.[9]

Determination of angle of repose:

The flowability was assessed by determination of the angle of repose (θ) using fixed funnel method. Angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and horizontal plane. Angle of repose has been used as indirect methods of qualifying powder flowability, because of their relation with interparticular friction.[10]

Post compression Study

Uniformity of thickness

Thickness and diameter of both core tablets and coated tablets were measured using a calibrated dial calipers. Three tablets of formulation were picked randomly and dimensions determined. It is expressed in mm and standard deviation was also calculated.

Weight variation test

To study weight variation 10 tablets of each pulse dose formulation were weighed separately and the test was performed according to the official method.

Hardness test

Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. Hardness of core tablets was determined using a validated dial type hardness tester. It is expressed in kg/cm^2. Three tablets were randomly picked from each batch and analyzed for hardness. The mean and standard deviation were also calculated.

Friability test

For each pulse dose tablet formulation, the friability of 10 tablets was determined using the Roche friabilator (Camp –bell Electronics, Mumbai, India).

F= [Initial weight – Final weight/Initial weight] ×100

Drug content

Ten tablets were weighed individually; these were placed in a mortar and powdered with a pestle. Accurately weighed powder sample equivalent to 20 mg of Capecitabine was transferred into a 20 ml volumetric flask and made up to volume with phosphate buffer of pH 6.8. The solution was

then filtered, suitably diluted with distilled water and absorbance was measured at 240 nm UV-Visible Spectrophotometer.

Concentration of polymers

Enteric coated tablet were prepared by using different ratios of material like Eudragit S-100, Eudragit L-100 and Guar gum. Required quantities of polymers were mixed proper. Concentration of polymers was shown in Table 2.

Table 2: Concentration of polymers

Sr. No.	Polymer	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Eudragit S-100(mg)	50	75	50	100	50	125	25	100	-
3	Eudragit L-100(mg)	50	50	75	50	100	25	125	-	100
4	Guar gum (mg)	50	25	25	-	-	-	-	50	50
5	Total(mg)	150	150	150	150	150	150	150	150	150

***In-vitro* Drug Release Studies of Tablet**

Drug release studies of matrix tablets were carried out using USP XXIII dissolution rate test apparatus (Apparatus 2, 75 rpm, 37°C $\pm 2^0$C) for 2 hr in 0.1 N HCl (900 ml) as the average gastric emptying time is about 2 hr. Then the dissolution medium was replaced with pH-7.4 phosphate buffer (900 ml) for 3hr and then in pH 6.8 phosphate buffer (900 ml), tested for drug release up to complete drug release. At the end of the time period 10 ml of the samples were taken and analyzed for content. A 10 ml volume of fresh and filtered dissolution medium was added to make the volume after each sample withdrawal. Sample was analyzed using UV spectrophotometer.

Stability Studies

The stability study of the formulations was carried out according to ICH guidelines at 40 ± 2^o C/75 ±5% RH for one month by storing the samples in stability chamber. The final formulation was packed in suitable packing like blister and strip packs and then they will be kept at different temperature, humidity conditions and the samples will be analyzed for their physical and chemical properties.

RESULTS AND DISCUSSION

Physical description of drug

Capecitabine was a white to off-white crystalline powder. The melting point of Capecitabine in literature is 110-121^{0}C after estimation by capillary method it was found to be 120^{0}C.Which indicates purity of drug sample. Capecitabine is soluble in water, ethanol, methanol, DMSO and DMF. Solubility analysis is important because the drug has to be soluble in the solvents and also in the dissolution medium.

Drug Excipients Compatibility Study

For identification FTIR of the obtained Capecitabine sample was conducted and shown in fig. no. 1. After comparing the FTIR spectra of drug sample and by comparing the spectral peaks in the spectra with standard reference spectra it was confirmed that the obtained sample is Capecitabine. The optimized batch showed identical spectrum with respect to the spectrum of the pure drug and polymers, indicating no chemical interaction or changes between the drug molecule and polymers during preparation of nanoparticles and Capecitabine was stable in preparation.

Differential Scanning Calorimetry (DSC)

The DSC technique provides qualitative and quantitative physicochemical status of drug which is reported in endothermic or exothermic process. The resulted thermal transition includes melting, decomposition and out gassing for change in heat capacity. Using DSC analysis of drug and polymer, the nature of the drug inside the polymer matrix can be assessed. In order to find out mechanism of zero order drug release. We first characterize the physical state within tablet. DSC thermo-gram of pure Capecitabine shows a sharp endothermic peak at 122.64^0C corresponding to its melting point. In DSC thermo-gram of formulation this endothermic peak is retained indicates that there is no interaction between drug and polymer and physical properties of drug retain was shown in figure 2.

Pre-compressional parameters

Granules of the formulations were subjected for various precompressional evaluations such as angle of repose, bulk and tapped density, compressibility index and Hausner's Ratio. Results of all the pre-compression parameters performed on the granules. The result of Compressibility index, Carr's index, Hausner's ratio and angle of repose given in Table No.3 and the results indicate that granules shows good flow property and excellent compressibility.

Table 3: Result of preformulation study of granules

Formulation	Angle of repose($_0$)	Bulk Density (gm/cm3)	Tapped Density (gm/cm3)	Hausner's ratio	Carr's index (%)
F1	25.12±0.98	0.646±0.006	0.735±.009	1.137±.003	12.09± 0.233
F2	24.78±.82	0.617±0.004	0.722±0.003	1.170±0.013	14.53 ±0.926
F3	26.89±0.80	0.634±0.005	0.720±0.008	1.136±0.022	11.9 9±1.739
F4	27.21±0.72	0.645±0.005	0.742±0.005	1.150±0.001	13.2 4±0.169
F5	25.62±0.53	0.652±0.012	0.740±0.003	1.134±0.021	11.8 9±0.562
F6	27.89±0.92	0.669±0.024	0.757±0.002	1.131±0.019	11.6 2±0.327
F7	26.58±0.94	0.654±0.011	0.728±0.003	1.130±0.009	12.1 6±1.202
F8	27.226±0.69	0.669±0.002	0.788±0.006	1.127±0.002	11. 29±0.324
F9	26.32±0.72	0.660±0.002	0.750±0.011	1.135±0.001	11.9 3±0.084

Post-compressional parameters

All the tablet formulations were subjected for evaluation according to various official specifications and other parameters. Shape, thickness, hardness, friability, weight variation, tablet dosage form and in vitro disintegration time.

Shape and appearance

Formulations prepared were randomly picked from each batch examined under lens for shape and in presence of light for color. Tablets showed standard concave surfaces with circular shape.

Uniformity of thickness

The mean thickness of tablets (n=3) of batch was 2.9±0.1mm. The standard deviation values indicated that all the formulations were within the range.

Weight variation test

The weight variation of the formulations is shown in Table No.16. All the tablets passed the weight variation test, i.e., average percentage weight variation was found within the Pharmacopoeial limits of ±10%.

Hardness test

Hardness or crushing strength of the tablets of all the formulations was studied. The mean hardness test results are tabulated in Table No. 4 The low standard deviation values indicated that the hardness of all the formulations was almost uniform and the tablets possess good mechanical strength with sufficient hardness .

Friability test

The results of friability value for all the batches was found obtained were found to be well within the approved range (<1%) in all the designed formulations. That indicated tablets possess good mechanical strength. The results are tabulated in Table 4 and 5.

Table 4: Result of preformulation study of core tablets

Formulation	Hardness (Kg/cm^3)	Weight Variation (%)	Friability (%)	Drug content Capecitabine	Thickness (mm)
F1	3.6±0.36	0.160±0.50	0.58±0.17	99.02±0.69	2.3±0.06
F2	3.5±0.30	1.65±0.14	0.64±0.15	97.13±0.41	2.1±0.17
F3	3.8±0.38	0.450±0.72	0.59±0.12	102.43±0.35	2.5±0.12
F4	4.0±0.40	1.46±0.13	0.78±0.13	101.35±0.32	2.2±0.13
F5	3.7±0.37	0.68±0.58	0.65±0.09	98.18±0.67	2.0±0.10
F6	3.5±0.34	1.23±0.27	0.81±0.07	99.72±0.68	2.3±0.06
F7	3.9±0.39	1.20±0.42	0.70±0.05	101.35±0.34	2.2±0.13
F8	4.0±0.42	1.40±0.25	0.73±0.07	100.5±0.34	2.3±0.14
F9	3.8±0.40	0.64±0.36	0.49±0.02	99.32±0.36	2.5±0.12

Drug-content

The drug content of Capecitabine was given as table 4.

Table 5: Result of preformulation study of coated tablets

Formulation	Hardness(Kg/cm^3)	Wt. Variation%	Friability(%)	Thickness(mm)
F1	4.6±0.56	0.627±0.58	0.58±0.58	2.9±0.16
F2	4.5±0.51	0.499±0.52	0.64±0.68	3.2±0.25
F3	4.8±0.62	0.599±0.50	0.59±0.57	3.3±0.28
F4	4.5±0.52	0.729±0.62	0.78±0.74	3.1±0.24
F5	4.5±0.52	0.057±0.38	0.65±0.69	3.2±0.25
F6	4.6±0.57	0.599±0.49	0.81±0.79	3.4±0.27
F7	4.7±0.58	0.610±0.52	0.70±0.71	2.7±0.14
F8	4.5±0.50	0.627±0.58	0.73±0.73	2.8±0.15
F9	4.7±0.59	0.603±0.54	0.49±0.71	2.3±0.12

***In Vitro* drug release studies and kinetic study**

The ultimate aim was to develop a pH dependent and microbially triggered colon targeted drug delivery system for Capecitabine as certain the above fact. In – vitro release study were carried out in 0.1 N HCL pH 1.2 for 0 to 2 Hrs. in phosphate buffer pH 7.4 for 3 to 5 Hrs. and in phosphate buffer pH 6.8 for 6 to 10 hours was shown in fig. no. 3. Drug release was assessed by using USP dissolution test apparatus type I (Basket). 900 ml of dissolution medium maintained at 37±0.5^{0}C was used. Basket was rotated at 50 rpm for 10 hrs. An aliquot (9ml of samples) were withdrawn at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and10 hour's time intervals, replacing the same amount with the pre warmed fresh medium. The samples withdrawn were filtered and the amount of drug dissolved was analyzed by a UV spectrophotometer at 240 nm.

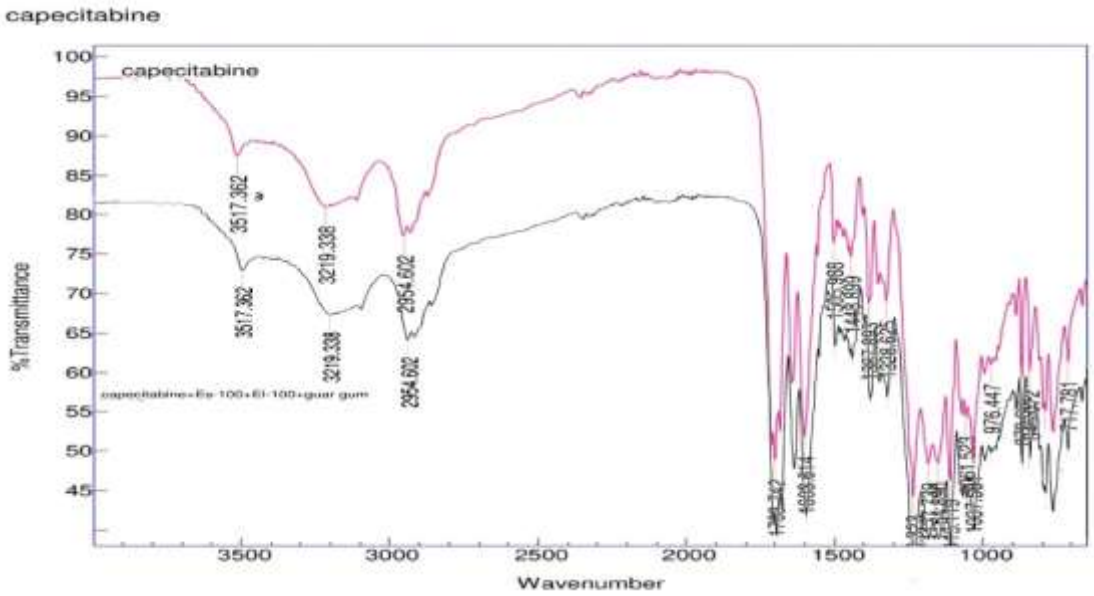

Figure 1: FTIR spectrum of pure Capecitabine and formulation.

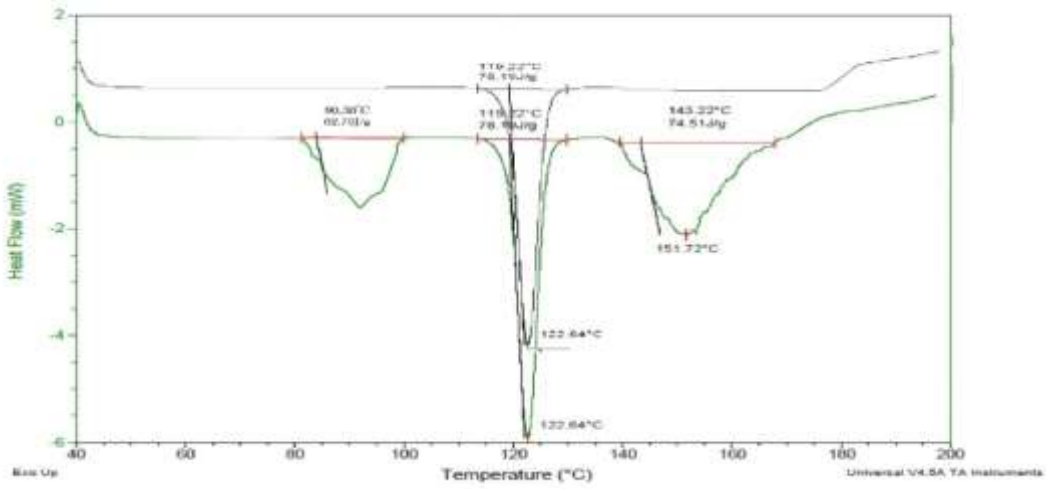

Figure 2: DSC graph of pure Capecitabine and formulation.

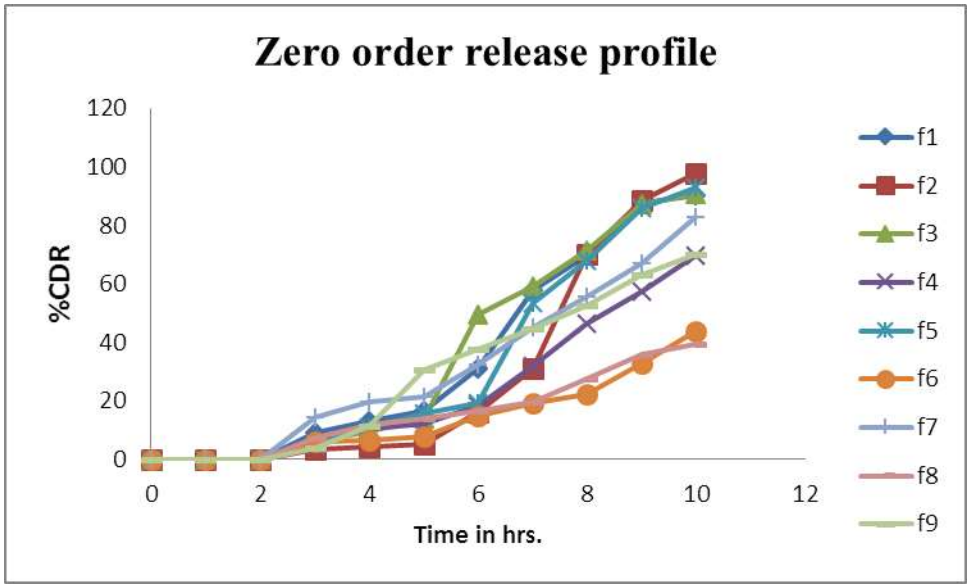

Figure 3: In Vitro drug release study of batch F1 to F9.

CONCLUSION

Capecitabine (antimetabolite) having shorter biological half-life (20 – 30mins), which is widely used in the treatment of colorectal cancer. The developed technology is targeting drug to the colonic region. The pH dependent and microbially triggered enteric coated tablet were developed by wet granulation method using Eudragit® S-100 for acid soluble coat due to its greater flexibility of film formation and desirable property of solubility below pH 7.0 and the guar gum is microbial triggering agent, was incorporated in immediate release layer. The coated tablets were coated with acid resistant polymer (Eudragit®L-100). The natural polymers guar gum and Lactose showed synergistic effect of drug release for a period of 10hours. The enteric resistance of

Eudragit® S-100 was dependent on the concentration of coating. The tablets show zero percent release in gastric environment. The use of pH dependent approach showed no drug release during the transit time in stomach. Finally, the release study concluded to develop and evaluate a pH dependent and microbial triggered colon specific drug delivery system of the Capecitabine for the treatment of colon cancer.

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