IJPSR (2019), Volume 10, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 September 2019; received in revised form, 18 October 2019; accepted, 22 October 2019; published 01 November 2019

IN-VIVO ROENTGENOGRAPHIC EVALUATION OF COLON TARGETED 5-FLUOROURACIL PELLETS

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Keywords:

5-Fluorouracil, Extrusion-spheronization, pH-sensitive, Time-dependent, Colon targeting, Pellets

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ABSTRACT: Objective: This study was an attempt to explore the latent of colon specificity approach utilizing pH-sensitive and time-dependent characteristics of polymers for defined colonic release of 5-Fluorouracil (5-FU). **Method:** The pellets were prepared by extrusion-spheronization method which is an industry-accepted method due to its ease of formulation. The prepared pellets were the matrix of 5-Fluorouracil with pH-sensitive and time-dependent polymers, *i.e.* Eudragit FS30D and Eudragit NM30D respectively. The changing pH media used for *in-vitro* release study of optimization batches for both the polymer concentrations. The Optical microscopy and Scanning electron microscope (SEM) was used to evaluate texture and surface morphology. **Results:** The $t_{10\%}$, $t_{30\%}$, $t_{50\%}$, and $t_{90\%}$ values for optimized formulation were found as 2.7 h, 6.2 h, 8.7 h and 18.2 h respectively. These values were extrapolated from *in-vitro* release *vs*. time plot which also indicated that the required lag time of 6-8 h was achieved. The *in-vivo* roentgenography or X-ray imaging study was used to confirm the lag time and transition path of colon targeted matrix pellets. **Conclusion:** The present study provides assurance for colon targeting of 5-FU pellets with industrially feasible processes. The combination of pH-sensitive and time-dependent polymers in development of pellets contributed to promising and precise drug release at colonic site. The *in-vivo* roentgenography study also further strengthen proposal of 6-8 h of lag time which was determined based on the *in-vitro* drug release study.

INTRODUCTION: According to the ASCO (American Society of Clinical Oncology), the proportion of patients diagnosed with colorectal cancer under the age of 50 was increased from 10% in 2004 to 12.2% in 2015 in the United States alone.

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DOI: 10.13040/IJPSR.0975-8232.10(11).5124-36

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(11).5124-36

In that also, nearly 52% of younger adults were diagnosed with more advanced stages of cancer (stage III/IV), whereas 40% in those older than 50 years [1]. Now a day, the scenario throughout the world is not different.

The several colonic diseases such as IBD (inflammatory bowel disease), CD (Crohn's disease), and ulcerative colitis, if not treated in their early stages, may lead to colorectal carcinoma [2-4]. 5-Fluorouracil (5-FU) is a widely prescribed drug for first-line chemotherapy of colorectal cancer. But this molecule is associated with few inherent limitations such as its instability in solution form,

photosensitivity, short half-life (8 to 20 min) and critical harmful effects if get exposed to undesired sites due to its high potency. Many attempts have been made in the last decade for developing the product which can release the 5-FU precisely in the desired colonic site without being released in other parts of the gastrointestinal tract (GIT) [5]. Earlier literature anticipated a range of approaches either of use of pH or time-dependent polymers or colonic microflora degradable polymers in matrix tablets. Very few of these attempts were able to make a correlation between *in-vitro-in-vivo* release of 5-FU due to its precincts. The reliability of use of the solely pH-dependent approach for colon targeting is very less as pH is highly-variable physiological parameter in IBD patients [6].

The fluoropyrimidine 5-FU is an antimetabolite drug that exerts its anticancer effects through inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA. Modulation strategies, such as co-treatment with leucovorin and methotrexate, have been developed to increase the anticancer activity [7]. The targeting of the drug with an appropriate release pattern to the site of action is more important for treating several chronic serious diseases [8]. Targeted delivery using multiparticulate system can be one of the more optimistic approaches that possibly will enhance its efficacy with reduced associated side effects [9].

However, intrinsic limitations and critical side effects limit the clinical application of 5-FU. Hence, the development of prominent colon-specific oral solid dosage form is an extreme dictate today. In this scaffold, multiparticulate system must be utilized due to its advantages such as predictable gastric transit time, minimum local irritation, enhanced surface area, reduced dose dumping and readily coatable which render them suitable for controlled drug delivery [10-14]. Prolonged residence time, specific pH level and microbial environment of colon represent the input essentials for colon targeting [15]. However, either of prodrug, time-dependent and/or pH-sensitive approach can be used to minimize the pre-colonic drug release [16]. A prodrug approach involving conjugation of drug with biodegradable polymers requires toxicological data and hence limits its application [17].

Zhang *et al*. used the time-dependent polymer coating of Eudragit RS100 to protect the drug release of famotidine from pellets at upper GIT and to integrate required lag time [18, 19]. However, the use of this approach was restricted by variation in gastric emptying time and intestinal transit time and need for advancement in manufacturing [20]. The drastic pH variation across the GIT, such as 1.2 in the stomach, 6.5 in the small intestine, and 7.2 in the colon, imply significance to deliver drug to the specific site using pH-sensitive polymer which mainly dissolves at pH above 6.8 [21-25]. However, pH in GIT may be altered depending upon age, sex, diet, and the disease condition, *etc*. Moreover, minute difference in pH between the small intestine and the colon makes it alone as a less reliable approach [26]. Zhao *et al*., have reported Total alkaloids of sophora alopecuroides (TASA) loaded pellets using the combination of time-dependent and pH-sensitive polymers as Eudragit RS30D and Eudragit S100, respectively to develop more reliable colon targeted system[27].

The main aim of this study was to develop a more reliable multiparticulate system comprising of matrix pellets of 5-FU by means of time-dependent and pH-sensitive polymers in combination for colon targeting. Eudragit FS30D was used for attaining precise colonic release and Eudragit NM30D for sustain release characteristics [28]. The extrusion and spheronization process, widely used in the industry, was selected for the preparation of the pellets as this process has scope for technological advancements and predictable and cost-effective scaling up capability. The optimized composition was used for *in-vivo* roentgenographic evaluation.

MATERIALS AND METHODS:

Materials: 5-fluorouracil was procured from Sigma Aldrich, Mumbai. IPCA Health Products Ltd. (Mumbai, India) provided Avicel PH 101 and Kollidon® VA 64 (vinylpyrrolidone-vinyl acetate copolymer). Eudragit NM30D and Eudragit FS30D were received as gift samples from Evonik Röhm GmbH (Darmstadt, Germany). Other excipients used to prepare pellets were of standard pharmaceutical grade and all chemical reagents of analytical grade. Other materials including barium sulfate were purchased from S.D. Fine-Chem. Ltd., (Mumbai, India).

Methods:

Preparation of Drug Loaded Pellets: The pelletization process used for preparing drug pellets was extrusion and spheronization technique. Table 1 represents the composition of pellets. The drug, Avicel PH 101 and Kollidon VA 64, were co-sifted through a 400 µm screen and mixed thoroughly. Kollidon® VA 64 is a vinylpyrrolidone-vinyl acetate copolymer and used as dry binder.

Eudragit NM30D and Eudragit FS30D dispersions were stirred for 15 min and were immediately added to the above mixture with uniform and slow speed. It was then kneaded to obtain a damp mass of required plasticity. The damp mass was passed through extruder and then extrudates were immediately spheronized to obtain pellets. Drug loaded pellets were dried in an oven at 40 °C for 24 h.

TABLE 1: COMPOSITION OF 5-FU PELLETS

Ingredients	Quantity (g)
5-FU	10.00
Avicel PH101	52.50
Kollidon® VA 64	2.50
Eudragit FS30D	20 (dry weight)
Eudragit NM30D	15 (dry weight)
Purified water (if required to maintain moisture level)	q.s.

The following parameters were kept constant for extrusion spheronization process, Extrusion Sieve: 1 mm, Extruder speed: 45 rpm, Radial plate of Spheronizer: 4.2 mm.

The process parameters related to pelletization were optimized from the preliminary trials to achieve uniform spherical pellets with sufficient hardness to avoid friability during subsequent processing.

Process Optimization of Pelletization: The extrusion-spheronization technique for pelletization was selected based on its known benefits compared to other processes. Extruder-20 (Anish Pharma) with sieve size of 1 mm operated at a speed of 45 rpm, and Spheronizer-250 (Anish Pharma) with plate size of 4.2 mm were used. The pelletization process was optimized by different spheronization speed and spheronization time for obtaining the pellets with desired characteristics.

To reduce the computational complexities, the above-mentioned components were eased to 2 independent variables namely,

Speed of spheronization (X1) = 600, 900, 1200 rpm

Time of spheronization (X2) = 10, 15, 20 min

The approximate appropriate levels of these independent variables were chosen from the data available from literature as well as the initial experimentation. The experimental grid was coded for ease of representation in **Table 2** and **Table 3**.

TABLE 2: TRANSLATION OF EXPERIMENTAL CONDITIONS INTO PHYSICAL UNITS FOR SPHERONIZATION

Levels	Factors (independent variables)		Response (dependent variables)			
	Spheronization speed (X_1)	Spheronization time (X_2)	Y_1	Y_2	Y_3	Y_4
-1	600 rpm	10 min	Aspect ration	Roundness	Carr's index	Pellet size
0	900 rpm	15 min				
+1	1200 rpm	20 min				

TABLE 3: EXPERIMENTAL DESIGN FOR SPHERONIZATION

Formulation code	X_1 (%)	X_2 (%)
T1	600	10
T2	600	15
T3	600	20
T4	900	10
T5	900	15
T6	900	20
T7	1200	10
T8	1200	15
T9	1200	20

Evaluation of Flow and Morphological Properties of Pellets: The prepared pellets were evaluated for parameters like friability, bulk density, tapped density, compressibility index (Carr's Index), and Hausner's ratio.

All the optimization batches were studied for morphological features like roundness, aspect ratio, pellet size, and shape by using photomicrograph (Optical microscope, Olympus CX 31).

Composition Optimization of Drug-Polymer Matrix Pellets: Selection of polymer concentration to be used in matrix formulation was necessary to achieve control drug release at the specific site of action and it is also important for targeting the

complete drug release in colonic site. The impact of concentration of Eudragit FS30D in the range of 15% to 25% (w/w) and Eudragit NM30D ranging from 10% to 20% (w/w) on the drug release at given time period ($t_{10\%}$, $t_{30\%}$, $t_{50\%}$, and $t_{90\%}$) was studied. These ranges were selected based on the prior experience and the drug release profile of preliminary batches. The optimized parameters which will remain constant during extrusion and spheronization are enlisted in **Table 4**. Variables and their levels along with experimental design are described in **Tables 5** and **6**.

TABLE 4: OPTIMIZED PARAMETERS FOR PELLETIZATION PROCESS

Parameters	Optimized values
Extrusion sieve size	1 mm
Extrusion speed	45 rpm
Spheronization plate size	4.2 mm
Spheronization speed	900 rpm
Spheronization time	20 min

TABLE 5: INDEPENDENT AND DEPENDENT VARIABLES AND CONCENTRATION OF POLYMERS

Levels	Factors (independent variables)		Response (dependent variables)			
	Eudragit FS30D Concentration (X_{M1})	Eudragit NM30D Concentration (X_{M2})	Y_{M1}	Y_{M2}	Y_{M3}	Y_{M4}
-1	15 %	10 %	$t_{10\%}$	$t_{30\%}$	$t_{50\%}$	$t_{90\%}$
0	20 %	15 %				
+1	25 %	20 %				

TABLE 6: EXPERIMENTAL DESIGN FOR POLYMER CONCENTRATIONS

Formulation code	X_{M1} (%)	X_{M2} (%)
F1	15	10
F2	15	15
F3	15	20
F4	20	10
F5	20	15
F6	20	20
F7	25	10
F8	25	15
F9	25	20

Friability*:* Friability was tested with 3 g of pellets placed in friabilator having 12 steel balls (0.445 g each) and tumbled at 25 rpm speed for 4 min.

Drug Content: Accurately weighed 500 mg of pellets were crushed in a dried mortar pestle and powder of pellets was dissolved in 50 ml with 0.01 N HCl. The sample was stirred for 15 min and filtered. Dilutions of solution were prepared and analyzed by UV-spectrophotometer (UV-Visible 2501 PC spectrophotometer (Shimadzu Co., Kyoto, Japan) at 266 nm.

Thermal Analysis: DSC (Differential Scanning Calorimetry: Mettler Toledo DSC 822e) was performed to measure the amount of heat energy absorbed or released by a sample, as it is heated, cooled or held at a constant temperature. About 10 mg of the sample was placed in DSC aluminium pans of 40 µl and it is sealed. An empty sealed pan is used as reference. Sample was run in the required temp range in inert atmosphere at a gas flow of 80 ml/min.

Powder X-Ray Diffraction Study: The sample was smeared over low background sample holder (amorphous silica holder) and fixed on the sample stage in goniometer. The instrument (Bruker Model D8 Advance) is set with B-B geometry. The current and voltage are set to 40 mV, and 35 mA and data have been collected.

Scanning Electron Microscopy (SEM): The drug-loaded pellets were evaluated for their surface morphology, texture, shape, and size. The sample was smeared on a small piece of adhesive carbon tape, which is fixed on a brass stub. The sample, then subjected to gold coating using sputtering unit (model: JFC1600) for 10 s at 10 mA of current. The gold coated sample placed in chamber of SEM (Jeol, JSM 6390LA) and secondary electron/Back Scattered electron images are recorded.

***In-vitro* Drug Release Study:** The extent of colon targeted pellets of 5-fluorouracil to protect the drug during the transit time in the gastro intestine region was assessed by mimicking mouth to colon transit. Drug release studies were carried out using USP XXIII dissolution basket method (100 rpm and 37 ± 0.5 ºC) in 900 ml pH 1.2 buffer solution for the initial 2 h, as the average gastric emptying time is 2 h, then the dissolution media is replaced with pH 7.4 phosphate buffer for 3 h, as the usual small intestine transit time is 3-5 h and dissolution was continued in phosphate buffer pH 6.8 up to 24 h to simulate the gastrointestinal environment as the

usual colon transit time is 20-30 h. 5 ml of aliquots were withdrawn and replaced with fresh medium at fixed time intervals. The sample was suitably diluted and analyzed for the percentage of drug release by UV spectrophotometer at the λ_{max} 266 nm [29]. All the measurements were performed in triplicates.

Statistical Analysis: The statistical analysis was done using one-way ANOVA with the assistance of Graph Pad Instat software, and $P<0.05$ was considered as a limit to indicate the statistical significance.

***In-vivo* Roentgenography or X-Ray Imaging Study:** The drug must be targeted to the mucosa of the terminal ileum for localized release to produce an efficient colon targeted drug delivery system. The release of drug in precolonic parts like stomach and upper small intestine is not acceptable as this will lead to premature absorption and consequent drug wastage as well as possible systemic side effects. It is very important to correlate the *in-vitro* performance of colon-specific formulation with *in-vivo* studies for ascertaining site-specificity because it is very difficult for formulations targeted to the ileocecal region, to endure and remain intact in the varied conditions of GIT. The *in-vivo* roentgenography or X-ray imaging study is very promising which is able to give the efficiency of colon specificity.

The animal experimental protocols were approved by the Institutional Animal Ethics Committee (Reg. No.535/02/a/CPCSEA/Jan.2002) with the approval letter no. IPER/IAEC/2015-16/06 and they were handled according to the code of ethics in research, training, and testing of the drugs. The animals were sourced from the animal house of the Institute of Pharmaceutical Education & Research (IPER) Borgaon (Meghe), Wardha, India.

The radio or X-ray imaging study on optimized formulation layered with barium sulfate was carried out. Two adult male New Zealand White strain rabbits weighing approximately 2–2.5 kg were used since this strain has been used previously for an *in-vivo* radio imaging study to assess the performance of the pH-dependent pulsatile drug delivery system [30]. Rabbits were kept on a standard diet and housed in separate standard cage racks with controlled humidity and temperature. For the study, rabbits were randomly divided into two groups and administered the two different pellet formulations. Group A and Group B contains one rabbit each and both were fasted overnight before start of the study.

The pellets without Eudragit FS30D and Eudragit NM30D were administered to the rabbit of group A through intubation tube followed by flushing of 25– 30 ml of water. If rabbit resists, it can be blown using rubber bulb. The matrix drug pellets with Eudragit FS30D and Eudragit NM30D were administered to rabbit of group B. During the entire study, care was taken that pellets were not chewed or vomited by rabbits.

X-ray image at 0 min was taken just before the administration of the dose to ensure the absence of any other radio-opaque material in the stomach and then subsequently photographs were taken at 2, 5, 6 and 8 h using Siemens X-ray machine, with 64 MAS and 63 KV techniques. The upright posture of rabbits was tried to maintain while taking X-ray images each time so that the formulation gets traced or captured in X-ray images with best possible resolution.

Stability Study: The stability study initiated by charging the sample in accelerated (40 ºC and 75% RH) for 3 mo and control sample at (25 ºC and 60 % RH) and at room temperature. The sample at accelerated condition was removed at 1, 2 and 3 mo intervals and analyzed for drug content and release profile.

RESULTS AND DISCUSSION: The matrix pellets of 5-FU with a pH-sensitive polymer and time-dependent polymer may have great implications to overcome the inherent problems of molecule, mainly photosensitivity, dose dumping, stability concerns as well as to target the specifically to colonic cells. As the pellets are transported rapidly from the upper GI tract, precolonic drug release from pellets is often controlled and certain areas of pathological interest in the lower GI tract can thus efficiently be targeted. The drug dosage requirement, as well as associated side effects, can be potentially minimized through the better distribution of pellets at target site.

5-FU is a highly potent molecule, and hence extrusion-spheronization process for pelletization was selected considering the efficiency, scalability and industrial acceptance. Preparation of drug-loaded pellets through the multiple-step process of extrusion-spheronization usually yields uniform and small-sized pellets with the advantage of incorporating proficiently higher levels of drug than other drug loading techniques. The manufactured pellets possess size distribution of 0.7 to 1.5 mm and were highly dense pellets. Moreover, the technique can be industrially adopted owing to its simplicity, rapidity and reproducibility [31-33].

Extrusion-spheronization is also known to produce spherical pellets that bestow desirable attributes including uniformity in size distribution, good flow, low friability and ease of coating [31, 33] However, the spheronization speed and the spheronization time, out of many other process variables, have a great influence on sphericity of the pellets, noticeably. Hence, spheronization process was optimized with respect to these variables and, expectedly, the chosen variables showed dominating authority on the spherical nature of the resultant pellets. With an increase in spheronization time and spheronization speed, pellets with very good roundness were observed. The selection of excipients was based on the results of preliminary trials. Avicel PH101 was used as a diluent, which also is known to aid the spheronization process and Kollidon VA64 as a dry binder. The resultant pellets were evaluated for their physical characteristics which are discussed in the following section.

Evaluation of Pellets of Process Optimization Batches: Flowability of the pellets is important factor while filling the pellets in capsules. Flow property is largely dependent on the particle size, shape, and density, amongst many other factors. Improvement in flow properties can be brought about by increasing the particle size and/or by producing spherical particles. The moisture content of the drug and excipients during the pelletization process also has an influence on the flowability and hence proper drying of pellets was ascertained.

The screen diameter determines the pellet size whereas the variables spheronization speed and time importantly contribute to the determination of pellet shape in the spheronization process [34]. Initial particle break-up of the extrudates is occurred by the speedy motion of the spheronizer while the collision frequencies and duration of the collision of the particles determine the roundness/sphericity of pellets. Sphericity is a key aspect of the coating and flow behavior of the pellets.

The lesser friability and certain hardness of the pellets are a prerequisite that reveals the mechanical strength of the pellets required for further processing such as capsule filling, packing, and transportation. Friability of pellets tested with steel balls was below 0.1%, signifying suitability of the pellets. Drug content or content uniformity shows that the drug is uniformly distributed in formulation which ensures the safety, efficacy, and quality of product. All the process optimization batches were evaluated for flow property and results of which are depicted in **Table 7**.

TABLE 7: FLOW PROPERTIES OF PELLETS

Trial	Angle of repose (°)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Hausner's ratio	Carr's index (%)
T1	35.85 ± 2.17	0.66 ± 0.07	0.81 ± 0.02	1.227 ± 0.004	18.519 ± 0.102
T2	32.71 ± 0.53	0.75 ± 0.09	0.88 ± 0.05	1.173 ± 0.006	14.773 ± 0.207
T3	31.40 ± 1.35	0.75 ± 0.04	0.84 ± 0.09	1.120 ± 0.007	10.714 ± 0.098
T4	28.36 ± 1.47	0.77 ± 0.04	0.86 ± 0.04	1.117 ± 0.009	10.465 ± 0.112
T5	24.52 ± 1.23	0.86 ± 0.03	0.95 ± 0.07	1.105 ± 0.003	9.474 ± 0.164
T6	24.23 ± 0.48	0.79 ± 0.05	0.83 ± 0.04	1.051 ± 0.002	4.819 ± 0.102
T7	34.92 ± 1.67	0.85 ± 0.09	0.98 ± 0.06	1.153 ± 0.009	13.265 ± 0.177
T8	32.17 ± 2.26	0.75 ± 0.07	0.85 ± 0.07	1.133 ± 0.003	11.765 ± 0.202
T9	25.98 ± 1.82	0.80 ± 0.05	0.84 ± 0.07	1.050 ± 0.004	4.762 ± 0.468

Each data point is mean ± SD for three values

The flow properties of pellets are the most important parameter for filling of pellets into empty capsule shell. The angle of repose values ranges from 24.23 ± 0.48 º to 35.85 ± 2.17 º. The values of angle of repose are rarely less than 20º and value up to 30º indicates reasonable flow potential. Above 40º, however, the powder flows with great difficulty.

The value of bulk density and tapped density ranges from 0.66 ± 0.07 gm/cm^3 to 0.86 ± 0.03 gm/cm^3 and 0.81 ± 0.02 gm/cm^3 to 0.98±0.06, respectively. The value of Carr's index below 15% indicates a powder which usually gives rise to excellent flow characteristics, whereas above 25 % indicate poor flowability. Hausner's Ratio (H) is an indirect index of ease of powder flow. The flow pattern of batch T6 and T9 was found to be excellent. Aspect ratio and roundness are important parameters for the characterization of pellets. Aspect ratio nearer to 1 and roundness nearer to 100% shows spherical pellets. The morphological characteristics of all optimization batches are as shown in **Table 8**.

TABLE 8: MORPHOLOGICAL PROPERTIES OF PELLETS

Batches	Shape	Aspect ratio	Roundness (%)	Pellet size (mm)
T1	Cylindrical /Rod	2.446 - 5.287	38.394 - 42.691	1.578 - 3.097
T2	Cylindrical /Rod	1.228 - 1.491	62.514 - 73.641	5.969 - 6.522
T3	Cylindrical + Dumb-bell	1.103 - 1.106	76.296 - 78.768	1.418 - 1.575
T4	Dumbbell + Oval	1.036 - 1.191	81.623 - 85.152	0.762 - 0.778
T5	Ellipsoid + Oval + Sphere	1 - 1.103	87.128 - 93.125	0.941 - 1.214
T6	Sphere	1 - 1.059	99.102 - 100	1.281 - 1.465
T7	Dumbbell + Ellipsoid	1.729 - 1.864	41.667 - 48.077	0.106 - 0.176
T8	Ellipsoid + Oval	1.206 - 1.218	63.763 - 67.473	0.14 - 0.69
T9	Oval + Sphere	1 - 1.068	97.072 - 99.807	0.112 - 0.239

Batch T6 showed the values of aspect ratio, and roundness 1 to 1.059 and 99.102 % to 100 % and batch T9 showed 1 to 1.068 and 97.072 to 9.807 respectively. But, the pellet size of batch T9 was found to be very fine, *i.e.* 0.112 mm to 0.239 mm as compared to batch T6, *i.e.* 1.281 mm to 1.465 mm. These coarser size pellets are desirable considering the flow and coating.

The Photo micrographic study also confirmed that batch T6 has more spherical and uniform pellets with a smooth surface than that of other batches. The comparative study of photomicrograph of all batches is as shown in **Fig. 1**. Thus, Batch T6 was selected as a final optimized batch and used for further purpose of study.

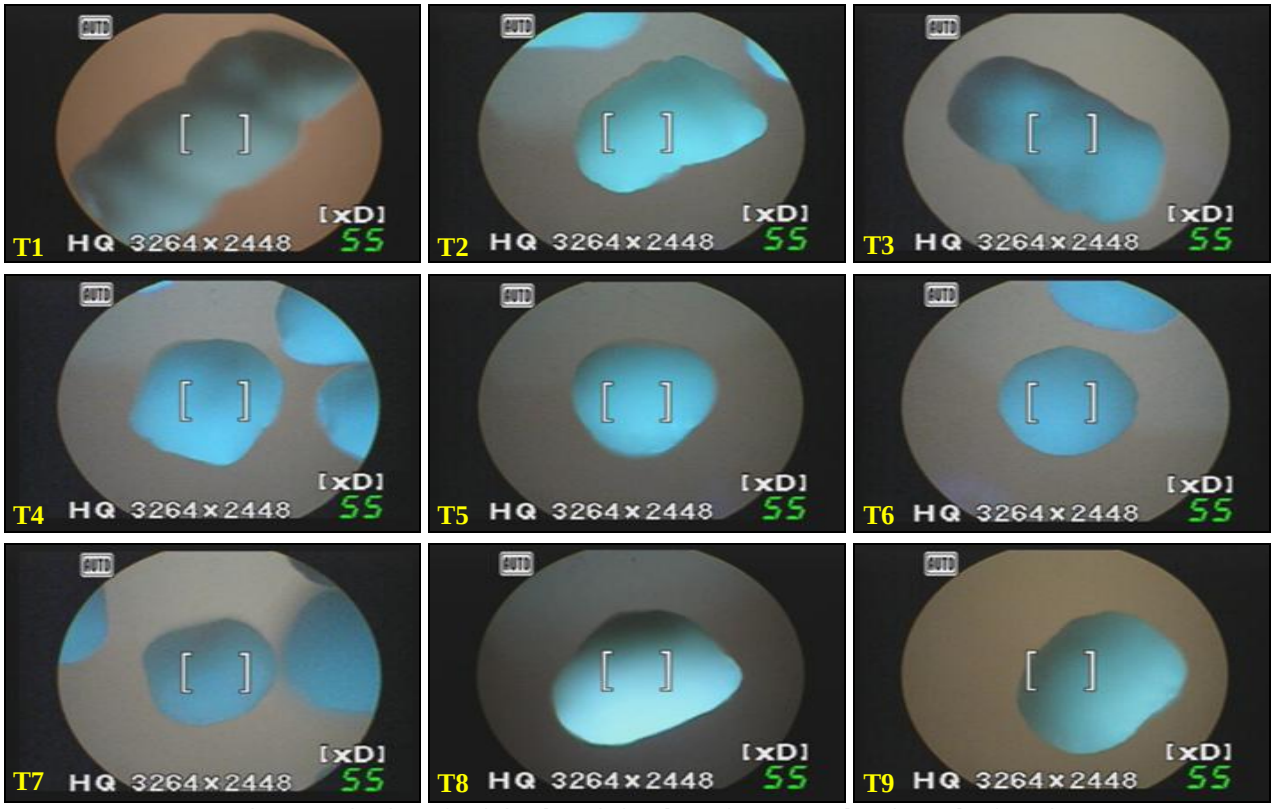

FIG. 1: PHOTOMICROGRAPHS OF ALL OPTIMIZATION BATCHES

Characterization of Drug-Polymer Matrix Pellets: Aqueous dispersion is used for pelletization purposes as recommended due to concern towards environmental protection. Eudragit NM30D is a neutral polymer consisting of an aqueous dispersion of poly (ethyl acrylate, methyl methacrylate) in 2:1 proportion and is non-toxic and does not produce any marked biological action. Its minimum film-forming temperature (MFT) is ~5 °C and allows formation of a water-insoluble, soft, and flexible film spontaneously without employment of plasticizer [35-37]. While Eudragit FS30D is an aqueous dispersion of an anionic and random copolymer based on methyl acrylate, methyl methacrylate, and methacrylic acid (7:3:1) with MFT of ~14 °C [28]. The optimization of concentrations of both the polymers, namely, Eudragit FS30D and Eudragit NM30D, in the matrix were carried out using already optimized pelletization process parameters, *i.e.* spheronization speed and spheronization time. The Eudragit FS30D was used in concentration of 15 %, 20% and 25% (w/w) whereas for Eudragit NM30D, 10%, 15% and 20% (w/w) concentrations were used.

DSC thermogram of the pure drug 5-FU **Fig. 2a** showed a characteristic exothermic peak at 289.76 °C which was within the range of melting point of the drug. A similar exothermic peak at 284.35 °C was exhibited by formulation composition containing 5-FU and excipients **Fig. 2b**. The observed melting point range was found to be in close proximity to the values reported. This study confirmed that there was no interaction between the drug and polymers used.

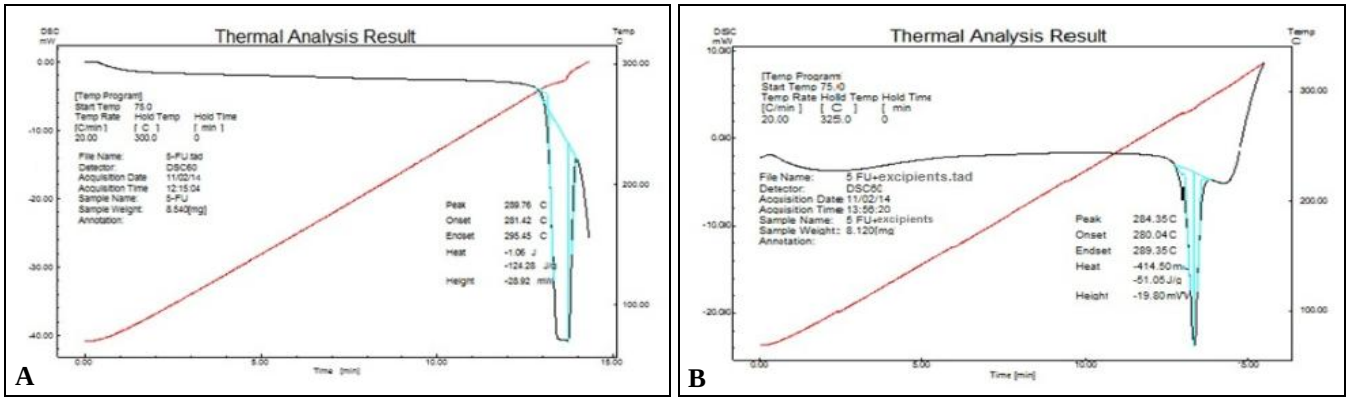

FIG. 2: A. DSC THERMOGRAM OF PURE DRUG 5-FU AND B. 5-FU AND EXCIPIENTS

The XRD peak mainly depends on the crystal size which indicates the crystalline nature at the particular value at 2θ range. In this study, pure drug 5-FU had shown a sharp single and the highest peak at 2θ of 28.1° that indicates its crystalline nature as depicted in **Fig. 3a**. The diffractogram for drug-polymer matrix had shown peaks at 22.6° and 16.1°, respectively which can be observed in **Fig. 3b**. It was found to be different from the diffractogram of pure drug, as noticed a minute decrease in the intensity of the peak, which can be attributed to the lower level of detection of the drug due to matrix formation of polymers, *i.e.* Eudragit FS30D and Eudragit NM30D, with 5-FU.

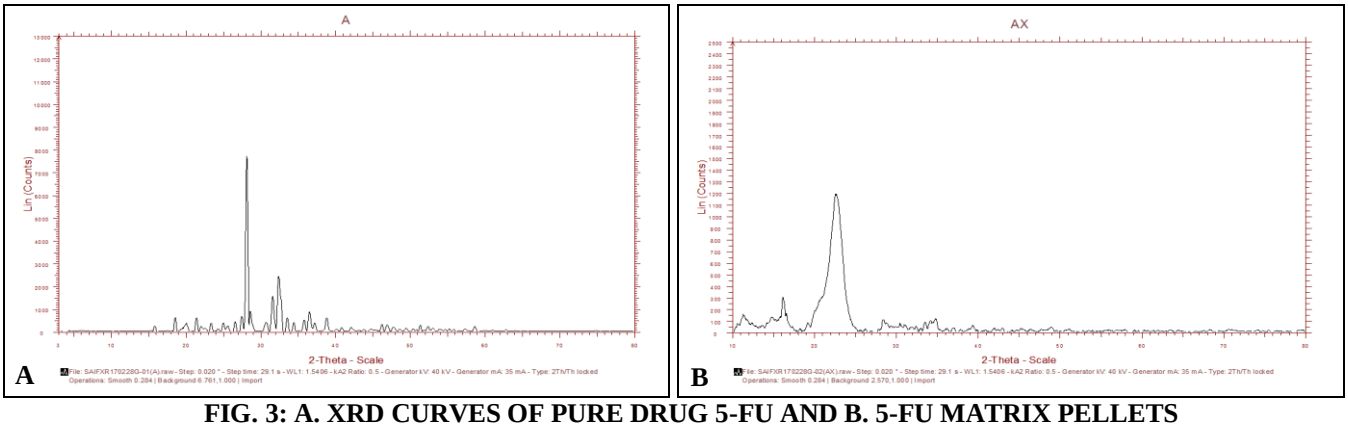

FIG. 3: A. XRD CURVES OF PURE DRUG 5-FU AND B. 5-FU MATRIX PELLETS

The low dose drug was dispersed at the molecular level and moreover the slight disappearance of the 5-FU peak indicates the entrapment of drug inside the polymeric matrix. Also the XRD peak for colon targeted polymeric matrix pellets was found to be of lower intensity and thus detection was found to be little difficult considering the low dose of 5-FU in pellets and due to presence of polymeric matrix.

SEM is one of the established and widely used techniques for the analysis of shape and size of different drug delivery systems [38]. SEM photographs of a cross-section of pellets revealed that the drug-polymer matrix was uniformly distributed throughout the core of pellets **Fig. 4a, 4b, 4c, 4d** and **4e**. The deformities like cracks and pores which generally are unavoidable and get formed during pelletization process. The surface texture was found to be smooth, free from any deformities or cracks **Fig. 4f** and **Fig. 4g**. This is due to critical process optimization of spheronization speed and time. The pellets were found to be round and spherical in shape which is the prerequisite for flowability of pellets **Fig. 4g** and **4h**.

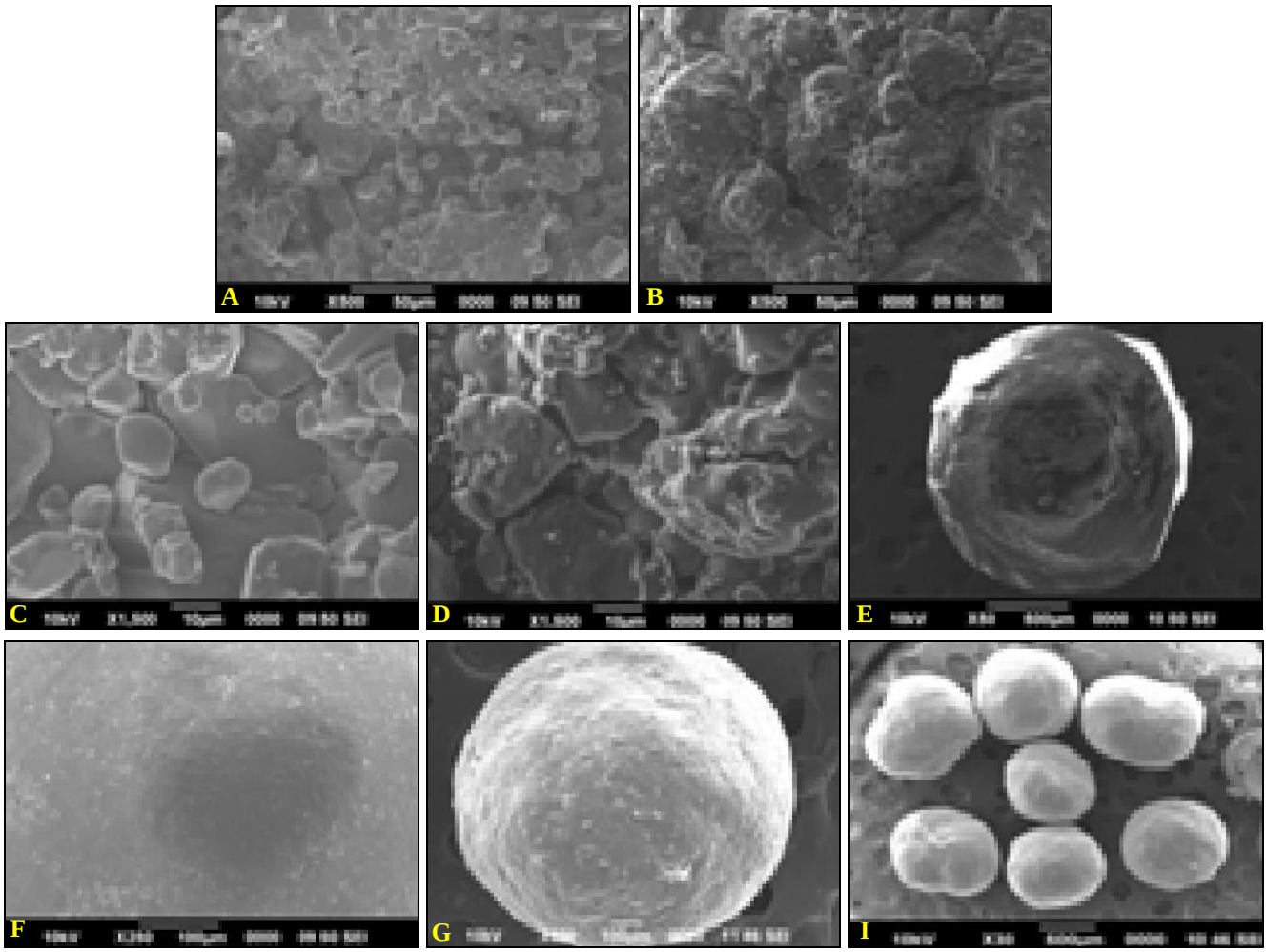

FIG. 4: A, B, C, D, AND E: REPRESENTATIVE SCANNING ELECTRON MICROGRAPHS DEPICTING THE DISTRIBUTION OF DRUG-POLYMER MATRIX AND F, G AND H: THE OVERALL APPEARANCE, SURFACE MORPHOLOGY AND SIZE OF PELLETS

In-vitro **Drug Release Study:** The mandatory requirement for an efficient colon targeted drug delivery system is to minimize the drug release in stomach at acidic pH (for the initial 2 h) and should show maximum release in the colonic pH at later phase. As the gastric transit time for similar drug delivery formulations is 2 h and for small intestine is 3 h[39], the drug release was designed to change the pH of the media at certain time intervals accordingly.

The pH and residence time at different parts of the GI tract are the prevailing factors for targeted colonic drug release. The Eudragit FS30D exhibits

the peculiar pH-sensitivity and is responsible for protection to the pellets in gastric environment whereas Eudragit NM30D is pH-independent, insoluble polymer, the permeability of which increases with time, and so provides an appropriate choice for the development of oral sustained release dosage forms. The pH in the GIT varies to a great extent depending up on-site and state of fasting or fed (in stomach, Fasted: 1.5-3 Fed: 2-5) in small intestine (Duodenum, Fasted: ≈6.1 and Fed: ≈5.4, Jejunum Fasted: 6-7, Ileum: ≈7-8).

In large intestine also it varies significantly (Cecum: 6.4, Ascending colon 5.7, Transverse colon 6.6, Descending colon and in rectum 7.0). Hence, in the combination of pH and time-dependent systems, if in case pH-sensitive polymer (Eudragit FS30D) could not achieve release in colon due to pH variability, then time dependant polymer (Eudragit NM30D) contribute for controlling the release. The reason behind using changing pH media methods for dissolution studies was the drastic changes in the colonic pH in case of colonic diseases, and thus to simulate the pH conditions of GIT.

Fig. 5 shows 5-FU release profiles in simulated gastric fluid (SGF, 0.1 N HCl, pH 1.2) for 2 h and in pH 7.4 (phosphate buffer) for 3 h and pH 6.8 for further up to 24 h. The matrix pellets with 15 % concentration of Eudragit FS30D (F1, F2, and F3) showed drug release only up to 9 h, 10 h, and 12 h, respectively. The increase in release time from F1 to F3 batches is attributed to increase in concentration of Eudragit NM30D as sustain release characteristics. Batch F4 with 10% of Eudragit NM30D and 20% of Eudragit FS30D could sustain drug release up to 18 h. However, for efficient colon targeting the prerequisite is to minimize drug release in first 2 h and almost complete release up to 24 h [40]. Hence, results figure out the inappropriateness of concentrations of polymer in F1-F4 batches. Batches F5 and F6 showed 5-FU release more than 90 % in 24 h, while batches F7-F9 could not completely release the drug within 24 h. The higher level of Eudragit FS30D (25%) in batches F7, F8 and F9 were accountable for retardation of drug release. Accordingly, the focus for getting optimum polymer concentration were centered on F5 and F6 batches, which showed 99.17 ± 0.13% and 92.26 ± 0.27% of 5-FU release in 24 h, respectively. Another important aspect for colonic drug delivery is minimal drug release in the stomach, and both the batches (F5 and F6) offered less than 5% of 5-FU release in 2 h signifying the gastroresistancy of combination of Eudragit FS30D at 20% concentration and Eudragit NM30D at 15% and 20% concentration levels. On the other hand, batch F5 is anticipated as superior over F6 (both with 20% Eudragit FS30D concentration) since not only did batch F5 exhibited almost complete drug release in 24 h along with comparable release in initial 2 h as compared to F6, but also the cost of the polymer (that definitely going to have a significant role for large scale production of pellets) can be considered as an important selection criterion. Additionally, batch F5 showed only 28.83 ± 0.17% drug release in 6 h, which is the time required for pellets to reach colon, and hence precolonic release was minimized together with maximum release coinciding with colonic residence [29].

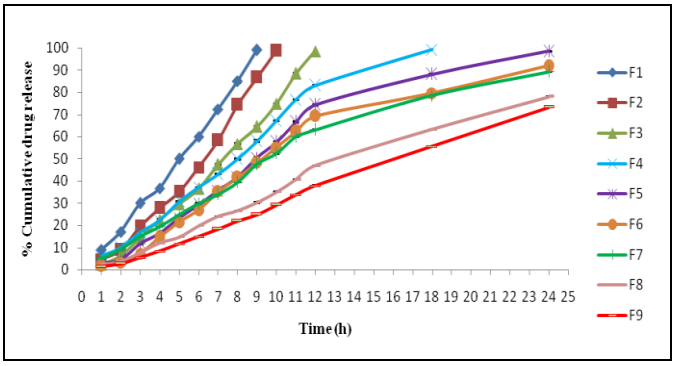

FIG. 5: *IN-VITRO* PERCENT CUMULATIVE DRUG RELEASE GRAPH OF FORMULATIONS F1 TO F9 CONTAINING 5-FU IN pH 1.2 (2 H), IN pH 7.4 (2-5 H) AND IN pH 6.8 (5-24 H) MAINTAINED AT 37 ± 0.5 °C (MEAN ± SD, N = 3)

The rate of drug release was decreased with enhance in the concentration of Eudragit NM30D imparting water-insolubility and diffusion-release characteristics over the entire pH range. It was also realized that higher concentration of Eudragit FS30D shaped larger lag time compared to the lower concentration levels. Accordingly, the optimum formulation (F5) was selected based on the feasibility and cost-effectiveness of large scale production, and lag time of 6-8 h (approaching the time when drug formulations have adequately been localized in the colon). The $t_{10\%}$, $t_{30\%}$, $t_{50\%}$ and $t_{90\%}$ extrapolated from the data which indicates the time required to get 10%, 30%, 50% and 90% release.

The $t_{10\%}$, $t_{30\%}$, $t_{50\%}$ and $t_{90\%}$ values for optimized batch F5 were 2.7 h, 6.2 h, 8.7 h and 18.2 h respectively.

***In-vivo* Study:** In recent times, the *in-vivo* performance of colon-specific drug delivery system was successfully accepted out by *in-vivo* roentgenography or X-ray imaging studies for optimized barium sulphate loaded pellets. In the present investigation, *in-vivo* radio imaging study conducted in rabbits. Here, 6-8 h of lag time which was observed for *in-vitro* release was then confirmed using a radio imaging technique for product performance in rabbits. The radiographic images are depicted in **Fig. 6**. **Fig. 6a** to **6e** represents the images of group A rabbit which was administered the pellets prepared without the use of Eudragit FS30D and Eudragit NM30D. The rationale of incorporation of this dosing is for comparing the study effect with non colon targeted or conventional dosage system. The images in **Fig. 6f** to **6j** are for group B rabbits who received matrix pellets containing Eudragit FS30D and Eudragit NM30D which were used for achieving colon targeting. The images taken at 0 h of study confirmed the absence of any other radioactive materials in the GIT of rabbit prier to pellets administrations **Fig. 6a** and **6f**. The pellets have reached the stomach around 2 h in both rabbit groups, as indicated with circular marks in **Fig. 6b** and **6g**. However, absence of pellets in **Fig. 6c, 6d,** and **6e** indicated that pellets were reached in intact form up to stomach only and followed the disintegration of pellets. The **Fig. 6h, 6i** and **6j** revealed that pellets were intact during transit from stomach to small intestine and then up to colonic region. These images were taken at 5 h, 6 h, and 8 h respectively after pellets administration to group B rabbit. The optimum concentration of Eudragit NM30D and Eudragit FS30D were played important role to protect the matrix pellets in its structural integrity and shape in stomach and small intestine and precisely reach the colon. At the end of 8 h barium sulfate was released from pellets and confirmed the arrival in the ileocecal region.

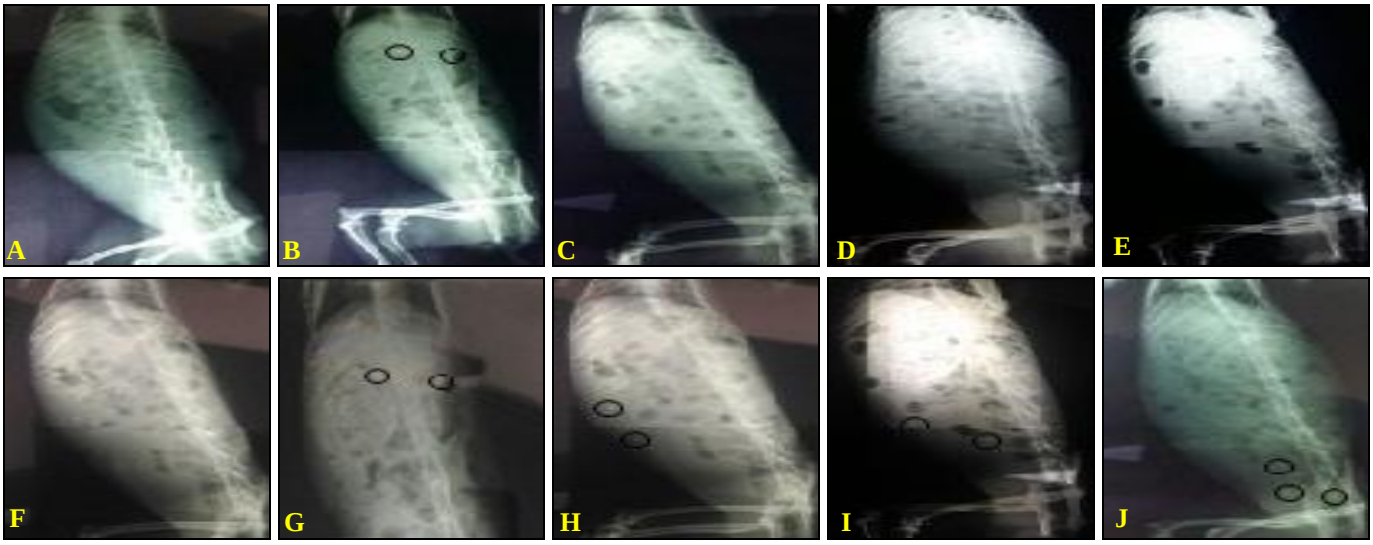

FIG. 6: ROENTGENOGRAPHIC OR X-RAY IMAGES INDICATING TRANSIT OF BOTH TYPES OF PELLET FORMULATIONS THROUGH GIT OF RABBIT

The difference in the *in-vivo* images of both the rabbit groups suggests the efficiency of polymer concentration in matrix pellets. Mouth to ileum transit times of cell wall material in rabbits was found to vary between 6 to 8 h [41]. In another study, the transit of food in the form of individual particles through the stomach of rabbits was found to be in the range of 3-6 h, and shorter transit times were found in the small intestine (10-20 min in the jejunum and 30-60 min in the ileum) [42].

This approach achieved the reliable *in-vivo and in-vitro* correlation for colon targeted drug delivery by using pH-sensitive and time-dependent polymer system.

CONCLUSION: By and large, the study proposes a promising approach for the preparation of 5-FU matrix pellets for colon-specific delivery with outstanding *in-vitro-in-vivo* correlation and with scalable industrially acceptable manufacturing

process. To achieve the perfectly spherical pellets with good strength, extrusion-spheronization process was found to be an efficient pelletization method. The combination of pH-sensitive and time-dependent polymers in development of pellets provides assurance of effective colon targeting of 5-FU. The *in-vivo* roentgenography or X-ray imaging study further strengthen proposal of 6-8 h of lag time which was initially determined based on the *in-vitro* drug release study.

ANIMAL HANDLING ETHICS: Protocols of the animal handling performed in this study were approved by the Institutional Animal Ethics Committee (IAEC), a regulatory body under the purview of CPCSEA. CPCSEA - The committee for the purpose of control and supervision of experiments on animals is a statutory body formed by the act of Indian Parliament in the year 1960, under the prevention of cruelty to animals act in the aegis of Ministry of forest and animal welfare, India. Animals were handled according to the code of ethics in research, training, and testing of drugs.

The ethics committee approval number is IPER/IAEC/2015-16/06. All animal experiments comply with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines, EU Directive 2010/63/ EU for animal experiments.

ACKNOWLEDGEMENT: The authors are thankful to the Institute of Pharmaceutical Education & Research (IPER) Borgaon (Meghe), Wardha (Maharashtra), India, and Government College of Pharmacy, Aurangabad, (Maharashtra), India for providing necessary facilities. We are also grateful to Sophisticated Test and Instrumentation Centre, Cochin University of Science and Technology Kerala, India, for their valuable assistance with SEM studies.

CONFLICT OF INTEREST: The authors of this scientific publication report no conflict of interest in this work.

REFERENCES:

1. American Society of Clinical Oncology. Rates of Colorectal Cancer Diagnosis in Adults Younger Than Age 50. July 2019. Available at: https://www.ascopost.com /News/60271.
2. Eun RK and Dong KC: Colorectal cancer in inflammatory bowel disease: The risk, pathogenesis, prevention and diagnosis. World J Gastroenterol 2014; 20(29): 9872-81.
3. Michael W, Diana H, Marcus T, Achim W and Thomas H: Degradation studies of modified inulin as potential encapsulation material for colon targeting and release of mesalamine. Carbohydr Polym 2018; 199: 102-8.
4. Shipra K and Shobhit K: To study the ulceroprotective effect of leaves of *Moringa oleifera* on experimentally induced inflammatory bowel disease on animal models. Asian J Pharm Clin Res 2019; 12: 103-6.
5. Vemula SK: A Novel Approach to Flurbiprofen Pulsatile Colonic Release: Formulation and Pharmacokinetics of Double-Compression-Coated Mini-Tablets. AAPS Pharm Sci Tech 2015; 16(6): 1465-73.
6. Karthika C, Sureshkumar R and Suhail A: Formulation development and *in-vitro* evaluation of curcumin-loaded solid self-nanoemulsifying drug delivery system for colon carcinoma. Asian J Pharm Clin Res 2019; 12: 243-7.
7. Daniel BL, Paul HD and Patrick GJ: 5-Fluorouracil: mechanisms of action and clinical strategies. Nat Rev Cancer 2003; 3: 330-8.
8. Nagasamy VD, Shashikumar S and Rajeshkumar R: Sustained release microbeads of Ritonavir: In vitro and in vivo evaluation. Int J Appl Pharm 2019; 11: 189-98.
9. Sorina D, Simona RI, Andreea DL, Ștefania M, Elena D and Maria M: Efficiency of Multiparticulate Delivery Systems Loaded with Flufenamic Acid Designed for Burn Wound Healing Applications. J Immun Res 2019; 451310.
10. Ubgade S, Kilor V, Bahekar V and Ittadwar A: Formulation development of immediate-release pellets of Tadalafil: Solidification approach for nanosuspension. Int J Appl Pharm 2019; 11: 124-31.
11. Ramteke HK, Jadhav BV, Kulkarni NS, Kharat AR and Diwate SB: Preparation, evaluation and optimization of multiparticulate system of mebendazole for colon targeted drug delivery by using natural polysaccharides. Adv Pharm Bull 2015; 5(3): 361-71.
12. Kesisoglou F, Xia B and Agrawal NG: Comparison of Deconvolution-Based and Absorption Modeling IVIVC for Extended-Release Formulations of a BCS III Drug Development Candidate. AAPS J 2015; 17(6): 1492-00.
13. Gupta SK, Huneza A and Patra S: Formulation, development and *in-vitro* evaluation of Tramadol extended-release tablets. Int J Pharm Pharm Sci 2019; 11: 63-73.
14. Mane SR: Advances of Hydrazone Linker in Polymeric Drug Delivery. J Crit Rev 2019; 6: 1-4.
15. Gada S, Anandkumar Y and Setty CM: Drumstick mucilage microspheres for controlled release of lamivudine: design, optimization and *in-vitro* evaluation. Int J Pharm Pharm Sci 2019; 11: 60-8.
16. Amidon S, Brown JE and Dave VS: Colon-Targeted Oral Drug Delivery Systems: Design Trends and Approaches. AAPS Pharm Sci Tech 2015; 16(4): 731-41.
17. Sinha VR and Kumria R: Microbially triggered drug delivery to the colon. Eur J Pharm Sci 2003; 18: 3-18.
18. Gazzaniga A, Iamartino P, Maffione G and Sangalli ME: Oral delayed-release system for colonic specific delivery. Int J Pharm 1994; 108: 77-83.
19. Zhang L, Chen DW and Gao ZB: Drug release mechanism of famotidine time-controlled release pellets. Acta Pharm Sin 2006; 41: 873-7.
20. Davis SS, Hardy JG, Taylor MJ, Stockwell A, Whalley DR and Wilson CG: The *in-vivo* evaluation of an osmotic device (osmet) using gamma scintigraphy. J Pharm Pharmacol 1984; 36: 740-2.

21. Sawarkar SP, Deshpande SG, Bajaj AN and Nikam VS: *In-vivo* evaluation of 5-ASA colon-specific tablets using experimental-induced colitis rat animal model. AAPS Pharm Sci Tech 2015; 16(6): 1445-54.
22. Zhang M and Merlin D: Nanoparticle-based oral drug delivery systems targeting the colon for treatment of ulcerative colitis. Inflam Bowel Dis 2018; 24(7): 1401-15.
23. Charbe NB, McCarron PA, Lane ME and Tambuwala MM: Application of three-dimensional printing for colon targeted drug delivery systems. Int J Pharm Invest 2017; 7(2): 47-59.
24. Gulbake A, Jain A, Jain A, Jain A and Jain SK: Insight to drug delivery aspects for colorectal cancer. World J Gastroenterol 2016; 22(2): 582-99.
25. Belali N, Wathoni N and Muchtaridi M: Advances in orally targeted drug delivery to colon. J Adv Pharm Technol Res 2019; 10(3): 100-6.
26. Maharshi P, Singh SK, Gulati M, Yadav AK, Garg V, Kumar B: A novel three-pronged approach for colon delivery of sulfasalazine: concomitant use of ph-responsive, microbially triggered polymers and liquisolid technology. Curr Drug Delivery 2018; 15(7): 1038-54.
27. Lijun S, Liping L, Xiaoying S, Honglang C, Shumin Z and Wenchang Z: Optimizing pH-sensitive and time-dependent polymer formula of colonic pH-responsive pellets to achieve precise drug release. Asian J Pharm Sci 2019; 14: 413-22.
28. Moghimipour E, Rezaei M, Kouchak M, Fatahiasl J, Angali KA, Ramezani Z and Amini M: Effects of coating layer and release medium on release profile from coated capsules with Eudragit FS 30D: an *in-vitro* and *in-vivo* study. Drug Dev Ind Pharm 2018; 44(5): 861-7.
29. Sidramappa MC and Baswaraj VR: Formulation, *in-vitro* drug release and *in-vivo* human X-ray investigation of polysaccharide based drug delivery systems for targeting 5-fluorouracil to the colon. Braz J Pharm Sci 2013; 49: 263-73.
30. Mastiholimath VS, Dandagi PM and Jain SS: Time and pH-dependent colon-specific, pulsatile delivery of theophylline for nocturnal asthma. Int J Pharmaceut 2007; 328: 49-56.
31. Wang Q, Guo M, Adu-Frimpong M, Zhang K, Yang Q and Toreniyazov E: Self-micro-emulsifying controlled the release of eugenol pellets: preparation, *in-vitro / in-vivo* investigation in beagle dogs. AAPS Pharm Sci Tech 2019; 20(7): 284.
32. Lu XY, Xu W, Ye M, Chu KD, Xu W and Wang XY: Preparation of *Rhus chinensis* total phenolic acid pellets by the extrusion-spheronisation method. Zhongguo Zhong Yao Za Zhi 2019; 44(13): 2785-91.
33. Barbosa EJ and Ferraz HG: Gellan gum and polyvinylpyrrolidone (PVP) as binding agents in extrusion/ spheronization pellet formulations. Acta Pharm 2019; 69(1): 99-109
34. Mooranian A, Zamani N, Mikov M, Goločorbin-Kon S, Stojanovic G and Arfuso F: Novel nano-encapsulation of probucol in microgels: scanning electron micrograph characterizations, buoyancy profiling, and antioxidant assay analyses. Artif Cells Nanomed Biot 2018; 46: 741-7.
35. Li F, Zheng X, Bao Y, Chen T, Zeng J and Xu X: Fenofibrate modified-release pellets with lag phase and high oral bioavailability. Drug Des Dev Ther 2018; 13: 141-51.
36. Zhao X, Li G, Zhang L, Tao X, Guan T and Hong M: Preparation and evaluation of nicotinic acid sustained-release pellets combined with immediate release simvastatin. Int J Pharm 2010; 400: 42-8.
37. Todoran N, Ciurba A, Al Hussein S, Lazar L, Ion V and Antonoaea P: Development of a modified-release pellets formulation--with metoprolol tartrat and kinetic aspects of in vitro release. Rev Med Chir Soc Med Nat Iasi 2014; 118(4): 1143-9.
38. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh FD, Javanmard R and Dokhani A: Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics 2018; 10: 57.
39. Kotla NG, Gulati M, Singh SK and Shivapooja A: Facts, fallacies and future of dissolution testing of polysaccharide based colon-specific drug delivery. J. Controlled Release 2014; 178: 55-62.
40. Bai XY, Yan Y, Wang L, Zhao LG and Wang K: Novel pH-sensitive hydrogels for 5-aminosalicylic acid colon targeting delivery: *in-vivo* study with ulcerative colitis targeting therapy in mice. Drug Delivery 2016; 23(6): 1926-32.
41. Gidenne T: Effect of fibre level, particle size and adaptation period on digestibility and rate of passage as measured at the ileum and in the faeces in the adult rabbit. Br J Nutr 1992; 67: 133-46.
42. Harcourt-Brown F and Harcourt-Brown N: Textbook of Rabbit Medicine. Oxford (UK): Butterworth Heinemann; 2002.

How to cite this article:
Bahekar JK and Wadher SJ: *In-vivo* roentgenographic evaluation of colon targeted 5-fluorouracil pellets. Int J Pharm Sci & Res 2019; 10(11): 5124-36. doi: 10.13040/IJPSR.0975-8232.10(11).5124-36.