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Original Article

FORMULATION DEVELOPMENT OF COLON TARGETED MESALAMINE PELLETS: *IN VITRO-IN VIVO* RELEASE STUDY

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ABSTRACT

Objective: This study was intended to investigate the potential of the colon specificity approach comprising of use of pH-sensitive and time-dependent polymers in combination for precise colonic release of Mesalamine or 5-Aminosalicylic acid (5-ASA).

Methods: The extrusion and spheronization method, preferably employed in industry for allowing high dose capacity to formulate, was used to prepare drug pellets. The Wurster coating technique used for aqueous coatings of Eudragit NE 40D as an inner coat and Eudragit FS30D as outer coat. The changing pH media used for *in vitro* release study of optimization batches for both the coating levels. A scanning electron microscope (SEM) was used to evaluate coating thickness and surface morphology.

Results: The pharmacokinetic parameters of formulation evaluated by *in vivo* study in rabbits revealed that the uncoated formulation released the drug too early in the gastrointestinal tract (GIT) with a mean C_{max} of 1205.28±0.37 µg/ml at 2 h after administration, whereas desired lag time was achieved in case of coated pellets exhibiting mean C_{max} 465.94±0.21 µg/ml and t_{max} of 8 h.

Conclusion: The *in vitro* and *in vivo* release study divulge the reliability of approach involving the use of pH sensitivity and time dependency of polymer for drug release in a single formulation for the treatment of colonic diseases. Hence, the present study provides constructive results for colon targeting of 5-ASA pellets with industrially feasible processes.

Keywords: pH-sensitive, Time-dependent, Wurster coating, Colon targeting, Mesalamine, Pellets

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INTRODUCTION

Recently, the occurrence of colon related diseases has increased greatly all over the world [1]. Mesalamine or 5-Aminosalicylic acid (5-ASA) is being more preferably used for the treatment of several colonic diseases such as IBD (inflammatory bowel disease), CD (Crohn's disease) and ulcerative colitis [2]. All these diseases, if not treated in their early stages, may lead to colorectal carcinoma [3]. Many attempts have been made in the last decade for developing the effective formulation to release the 5-ASA precisely in the colon without being released in other parts of the gastrointestinal tract (GIT) to avoid the premature absorption and loss of drug [4]. Prier literature projected a range of approaches either of colonic microflora degradable or use of pH or time-dependent polymers in matrix tablets. Very few of these attempts were able to make a correlation between *in vitro-in vivo* release of this molecule due to its drastic biovariavbility. Use of the solely pH-dependent approach for colon targeting has limitation as pH is highly-variable physiological parameter in IBD patients [5]. 5-ASA acts as an anti-inflammatory agent through inhibition of cyclooxygenase and by decreasing prostaglandin production and blocking the production of interleukin-1 (IL-1) and tumor necrosis factor-a (TNF-a) [6-7]. Several chronic serious diseases might be capable of being treated more effectively if drugs were targeted on the site of action with an appropriate release pattern [8]. Targeted delivery of the 5-ASA using multiparticulate system can be one of the more hopeful approaches that could enhance its efficacy with reduced associated side effects [9].

However, high biovariability and high dose limits the clinical application of 5-ASA. Hence, there is a pressing need to develop colon-specific oral solid dosage form. In this framework, pellets offer advantages such as predictable gastric transit time, minimum local irritation, enhanced bioavailability, 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 key elements for colon targeting [15]. However, the limitation is the pre-colonic drug release which can be circumvented by employing either prodrug, time-dependent and/or pH-sensitive approach [16]. A prodrug approach involving conjugation of drugs with biodegradable polymers requires toxicological data and hence limits its application [17]. The Famotidine time-controlled release pellets were developed by Zhang *et al.* using time-dependent polymer coating of Eudragit RS100 to protect drug release at upper GIT and incorporate required lag time [18, 19]. However, variation in gastric emptying time and intestinal transit time and need of advancement in manufacturing restricts the use of this approach [20]. One more approach exploits 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, and hence imply significance to deliver drug to the specific site using pH-sensitive polymer [21-23]. Polymers that mainly dissolve at pH above 6.8 can be employed for colon targeting e. g., polyvinyl acetate phthalate, cellulose acetate phthalate and copolymers of acrylic and methacrylic acid (Eudragit FS 30D, S100 and L100) [24, 25]. However, pH in GIT may be altered depending upon the disease condition, diet, age, sex, etc. Moreover, small difference in pH between the small intestine and the colon makes it alone as a less reliable approach [26]. Kadam *et al.* have reported theophylline loaded pellets using the combination of time-dependent and pH-sensitive polymers as Eudragit RL100 and Eudragit S100, respectively to develop more reliable colon targeted system [27].

Hence, the objective of this study was to develop a more promising, time-dependent and pH-sensitive combination-based 5-ASA loaded pellets for colon targeting. Eudragit FS30D (with superior site specificity than commonly employed Eudragit S100) was used for outer coating [28], whereas Eudragit NE40D as an inner coating material produced sustain release characteristics. The Wurster coating process was used due to its industrial acceptance, technological advancements, predictable and cost-effective scaling up capability and higher drying capacity for aqueous coating. The pharmacokinetic parameters were evaluated on optimized composition.

MATERIALS AND METHODS

Materials

5-aminosalicylic acid was procured from Sigma Aldrich Co. IPCA Health Products Ltd. (Mumbai, India) provided Avicel PH 101, Hypromellose and polyvinylpyrrolidone (PVPK30). Eudragits

NE40D, Plasacryl T20, and Eudragit FS30D were received as gift samples from Evonik Röhm GmbH (Darmstadt, Germany). Other excipients used to prepare pellets and coating dispersion were of standard pharmaceutical grade and all chemical reagents of analytical grade. Other materials namely, isopropyl alcohol (IPA), Talc, Triethyl citrate (TEC) and Glyceryl monostearate (GMS) was purchased from SD Fine-Chem. Ltd., (Mumbai, India).

Methods

Preparation and optimization of drug-loaded pellets

Pellets were prepared by extrusion-spheronization technique. Extruder-20 (Anish Pharma) with a sieve size of 1 mm with use of speed 45 rpm and Spheronizer-250 (Anish Pharma) with plate size of 4.2 mm were used. The composition of pellets is listed in table 1. Drugs and all the excipients were sieved through a 400 µm screen and mixed thoroughly. Binder solution was prepared by dissolving 3 % w/w polyvinylpyrrolidone (PVPK30) in purified water under continuous stirring. PVP K30 solution was uniformly kneaded with above mixture 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.

Friability

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

Drug content

The drug content of Mesalamine was determined (sample size of 100 mg pellets) by using UV-Visible 2501 PC spectrophotometer (Shimadzu Co., Kyoto, Japan) at 302 nm.

Preparation of coating dispersion and coating of pellets

The new and innovative coating system additive, Plasacryl T20, was used during the preparation of Eudragit FS30D aqueous dispersion.

The dispersion of TEC and Plasacryl T20 (ready to add system is 20 % emulsion consisting Polysorbate-80 as a surfactant, triethyl citrate (TEC) as a plasticizer and glyceryl monostearate (GMS) as an antitacking agent) in water was added to polymer dispersion under stirring and then homogenized for 10 min. The resultant dispersion was passed through 0.5 mm sieve. The composition of inner coating and outer coating dispersion is shown in table 2.

Table 1: Composition of 5-ASA pellets

Ingredients	Quantity (g)
5-ASA	90.00
Avicel PH101	7.00
PVP K30 solution (5% w/w)	3.00 (dry weight)
Purified water (to maintain moisture level)	q. s.

The pelletization process was optimized by different spheronization speed and spheronization time to achieve uniform spherical pellets with sufficient hardness to avoid friability during subsequent processing.

Table 2: Composition of coating dispersions

Ingredients	Eudragit NE40D (inner coating)	Eudragit FS30D (outer coating)
TEC (with respect to dry polymer)	-	5 %
GMS (with respect to dry polymer)	5 %	-
Plasacryl T20 (with respect to dry polymer)	-	10 %
Polysorbate-80 (with respect to dry GMS)	40 %	-
Total solid content of spray suspension	20-25 %	20-25 %

Prepared coating dispersion was used for the coating of pellets using FBP (Model GPCG 1.1, Glatt). The process parameters related to FBP were optimized from the preliminary trials.

Table 3: Optimized FBP parameters for the coating process

Parameters	Optimized values
Spray type	Bottom spray
Bag type	Finger bag
Inlet temperature (°C)	35
Product temperature (°C)	25
Blower speed (Hz)	35
Pump speed (rpm)	3
Atomization pressure (bar)	1.2
Nozzle diameter (mm)	1

Table 4: Independent and dependent variables and the levels for coating

Levels	Factors (Independent variables)		Response (dependent variables)		
	Inner coating level (X_{C1})	Outer coating level (X_{C2})	Y_{C1}	Y_{C2}	Y_{C3}
–1	8 %	8 %	$t_{10\%}$	$t_{50\%}$	$t_{90\%}$
0	12 %	12 %			
+1	16 %	16 %			

Table 5: Experimental design for coating

Formulation code	X_{C1} (%)	X_{C2} (%)
F1	8	8
F2	8	12
F3	8	16
F4	12	8
F5	12	12
F6	12	16
F7	16	8
F8	16	12
F9	16	16

Optimization of the coating process

300 g of 5-ASA loaded pellets were coated using FBP. In-process sampling was done at regular intervals for observing coating morphology. At the end of coating, pellets were dried for 5 min by increasing temperature to 40 °C. Optimized coating parameters are enlisted in table 3.

Optimization of coating levels

Selection of inner coating level was necessary to achieve control drug release at the specific site of action and it is also important to optimize outer coating with Eudragit FS 30D for targeting the complete drug release in colonic site. The impact of inner and outer coating levels i.e. 8 %, 12 % and 16 % w/w (based on drug release profile of preliminary batches) on the drug release at given time period ($t_{10\%}$, $t_{50\%}$ and $t_{90\%}$) was studied. Variables and their levels along with experimental design are described in table 4 and 5.

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 aluminum 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 is set to 40 mV and 35 mA and data has been collected.

Scanning electron microscopy (SEM)

The coated pellets were evaluated for their surface morphology, shape, size and coating thickness. 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 the sputtering unit (model: JFC1600) for 10 s at 10 mA of current. The gold coated sample placed in the chamber of SEM (Jeol, JSM 6390LA) and secondary electron/Back Scattered electron images are recorded.

***In vitro* drug release study**

The changing pH media for delayed-release dosage form was used for studying the drug release from developed pellets. The dissolution test was conducted using USP type 1 apparatus (100 rpm, 37±0.5 °C, 900 ml). Initial drug release studies were conducted in 0.1N HCl (pH 1.2) for 2 h, subsequently in pH 7.4 (phosphate buffer) for 3 h and in pH 6.8 (phosphate buffer) till end of the test. 5 ml of aliquots were withdrawn at regular time intervals and replaced with fresh dissolution medium. The concentration of drug released was analyzed using UV spectrophotometer at wavelength of 301, 330 and 334 nm for pH 1.2, pH 7.4 and pH 6.8, respectively. All the measurements were performed in triplicate [29].

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* study**

For effective colon targeted drug delivery system, the drug must be targeted to the mucosa of the terminal ileum and colon for localized release. The release of drug in the stomach and upper small intestine is undesirable 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 due to the varied conditions in GIT of the formulations targeted to the ileo-cecal region for its site-specificity. Along with X-ray imaging study, pharmacokinetic study gives the reliability on colon specificity.

The animal experimental protocols were approved by the Institutional Ethical Committee for Care and Use of Laboratory Animal 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 and Research (IPER) Borgaon (Meghe), Wardha, India. The ethics committee approval number is: IPER/IAEC/2015-16/06.

A two-way randomized crossover study was planned with the use of six white New Zealand rabbits, approximately 2 kg. 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. On the first day of the study, the uncoated formulation was given to group I, while group II was received the coated pellets, and vice-versa on the second part of the study. Fifteen days of washout period was allowed between administrations. Rabbits were fasted for 12 h before the study and throughout the study but had free access to water. Pellet formulations (coated or uncoated) containing 50 mg/kg of 5-ASA were filled into hard gelatin capsules (size 4) and administered orally to rabbits using a cannula. To facilitate the ingestion of the capsule, 5 ml of water to be given to rabbits through a syringe after administration of the capsules. Blood samples (700–800 µl) was collected through a catheter inserted into the marginal vein of the ear of the rabbits, at pre-determined times (0, 1, 2, 3, 4, 6, 8, 12 and 24 h) and rapidly transferred into 1 ml blood collection tubes containing lithium–heparin. The plasma obtained after centrifugation of the blood samples at 13 800 rev/min for 15 min and kept at-80 °C until analysis [30].

Pharmacokinetics analysis

Plasma concentration of 5-ASA concentration in the plasma (µg/ml) as a function of the time of administration (in h) was plotted for both uncoated and coated pellets formulations. The maximum 5-ASA concentration in the plasma (C_{max}) is the arithmetic mean of values for the six rabbits in the two days of the study whereas the corresponding time (t_{max}) was read directly from the graph. The area under the 5-ASA plasma concentration-time curve ($AUC_{0\text{-}24h}$) was calculated using non-compartmental analysis (PK Solver 2.0).

Stability study

The stability study initiated by charging the sample in accelerated (40 °C and 75 % RH) for 3 mo and control sample at (25 °C 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

5-ASA pellets coated with a pH-sensitive polymer may have great implications to overcome the inherent problems of drug mainly biovariability concern as well as to target the specifically to colonic cells. Since pellets are transported rapidly from the upper GI tract, premature drug release from coated pellets is often restrained and certain areas of pathological interest in the lower GI tract can thus effectively be targeted. The better distribution of pellets at target site could potentially minimize the requirement of drug dosage and associated side effects as well.

5-ASA is a high dose molecule and hence the extrusion-spheronization process was selected considering the scalability and industrially acceptable approach for pelletization. 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 efficiently higher levels of drug than other drug loading techniques. The pellets produced possess size ranging between 0.5 and 2.0 mm and have high density. 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, sphericity of the pellets is often influenced by many process variables, noticeably, the spheronization speed and the spheronization time. Hence, the 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 PVP K30 solution as a binder. The resultant pellets were evaluated for their physical characteristics which are discussed in the following section.

Characterization of pellets

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. Also, the amount of moisture associated with the drug and excipients during the pelletization process has an influence on the flowability and hence proper drying of pellets was ascertained. The screen diameter determines the pellet size whereas pellet shape is determined by the variables in the spheronization process (importantly, spheronization speed and time) [34]. Initial particle break-up of the extrudates is triggered 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 factor for the coating and flow behavior of the pellets.

Friability test reveals the mechanical strength of the pellets required for further processing, including film coating. Certain hardness of pellets is a prerequisite for the coating process, especially in Wurster coating, to withstand the collisions in between particle-particle and particle with the walls of the instrument. Friability of pellets tested with steel balls was below 0.1 %, signifying suitability of the pellets in Wurster coating process. Drug content or content uniformity shows that the drug is uniformly distributed in formulation, which ensures the safety, efficacy and quality of product.

Coating of pellets and characterization of coated pellets

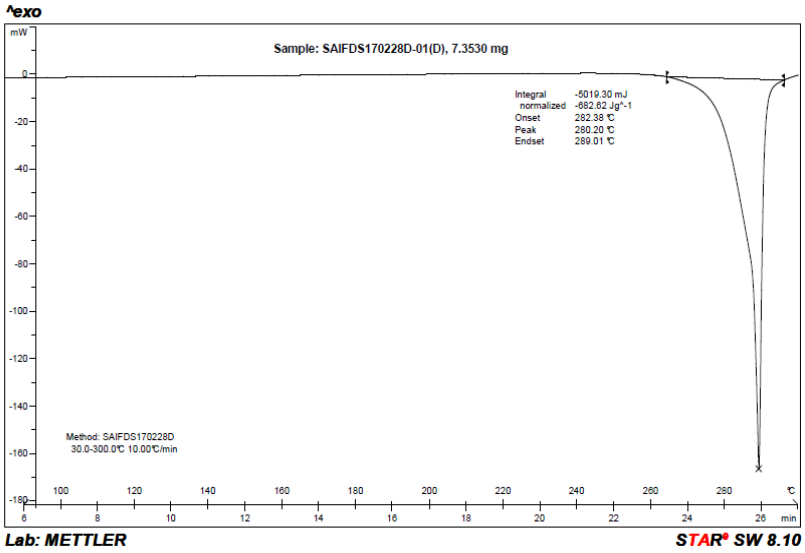
With concern towards environmental protection, use of aqueous-based solution/dispersion is recommendable for coating purposes. Eudragit NE40D is a neutral polymer consisting of an aqueous dispersion of poly (ethyl acrylate, methyl methacrylate) in 2:1 proportion and is non-toxic and does not produce 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 coating levels were optimized using the optimized batch of uncoated pellets to achieve inner and outer coat weight gain of 8 %, 12 % and 16 % (w/w) for respective Eudragit grades. The coating efficiency was determined which is expressed as the ratio of actual weight gained to the theoretical weight gain expected to be obtained after coating with respective coating levels. All the factorial batches (F1-F9) showed optimum coating efficiency for Eudragit NE40D as well as for Eudragit FS30D ranging from 98.11 % to 99.87 %.

DSC thermogram of the pure drug 5-ASA (fig. 1a) showed a characteristic exothermic peak at 280.20 °C, which was within the range of the melting point of the drug. Formulation composition containing 5-ASA and excipients exhibited a similar exothermic peak at 271.61 °C (fig. 1b). 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.

The 2θ values from the powder XRD studies for 5-ASA were found to be 14.991° and 26.922° as per the represented powder XRD curves in the (fig. 2a) and a sharp intense peak indicated the crystallinity of the drug. The 2θ value of formulation composition containing 5-ASA and excipients were found to be 15.030° and 26.965° (fig. 2b) indicating its crystalline nature. The 2θ value confirmed that the drug and the polymer existed in the crystalline state without any interaction and polymorphic change.

(a)



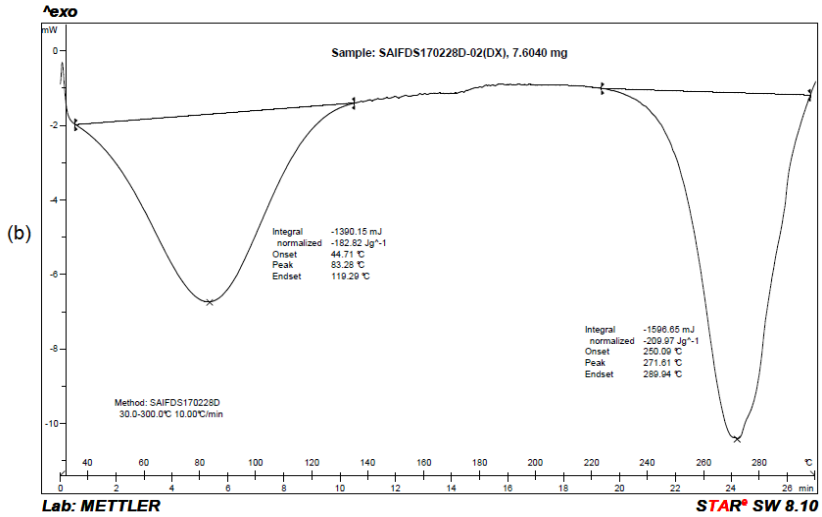


Fig. 1: DSC thermogram of pure drug 5-ASA (fig. 1a) and 5-ASA and excipients (fig. 1b)

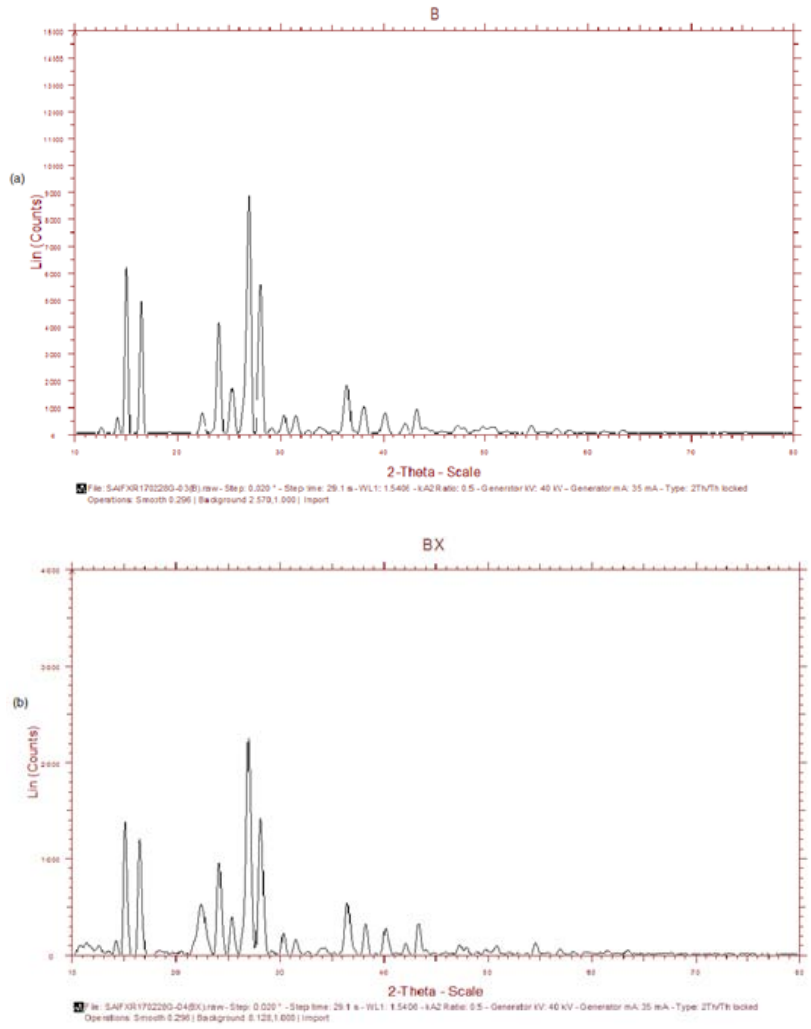


Fig. 2: XRD curves of pure drug 5-ASA (fig. 2a) and 5-ASA and excipients (fig. 2b)

SEM is one of the established and widely used techniques for the analysis of shape and size of different drug delivery systems (Danaei*et al.* 2018). SEM photographs revealed that the rough surface of core pellets (fig. 3a,) was almost getting covered after coating with improved surface texture and smoothness (fig. 3b and fig. 3c). The deformities like cracks and pores generally get formed during the pelletization process and are unavoidable. The coated pellets were larger in size as compared to uncoated pellets with about 1.0-1.22 mm of diameters. The average coating thickness estimated from SEM analysis was 73.91±2.0 µm (fig. 3d, 3e, and 3f).

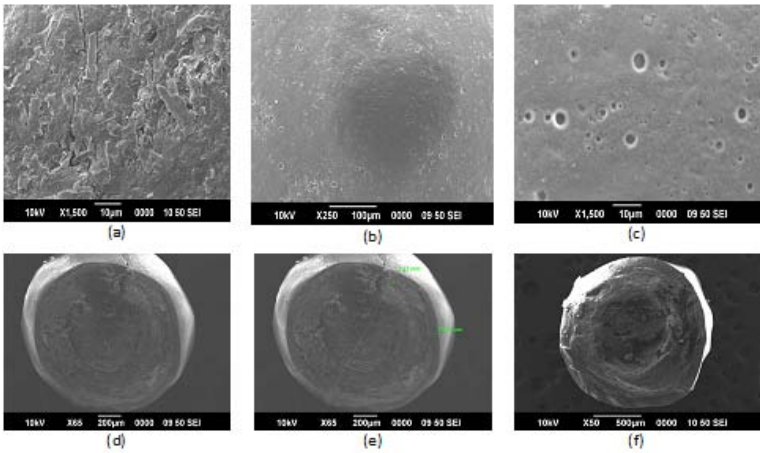


Fig. 3: Representative scanning electron micrographs depicting the overall appearance and surface morphology of core pellets (Fig.: 3a) and size and coating thickness of coated pellets (Fig.: 3b, 3c, 3d, 3e and 3f)

In vitro drug release study

For a colon targeted drug delivery system to be successful, its release pattern should have specific characteristics so that in stomach pH it should show minimum drug release (for the first 2 h) and should show maximum release in the colonic pH afterward. Since gastric transit time for similar drug delivery formulations is 2 h and for small intestine is 3 h [38], the drug release was designed to change the pH of the media at certain time intervals accordingly.

The governing factors for targeted colonic drug release include pH and residence time at different parts of GI tract. The peculiar pH-sensitivity of Eudragit FS30D is responsible for protection to the pellets in gastric environment, whereas pH independent permeability of water-insoluble film of Eudragit NE40D increases with time, and so provides an appropriate choice for the development of oral sustained release dosage forms. The pH in the GIT varies greatly 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 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 NE40D) contribute for controlling the release. As the pH of colon changes drastically in colonic diseases, and to simulate the pH conditions of GIT changing pH media method was employed for dissolution studies.

Fig. 4 shows 5-ASA 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. Pellets coated with Eudragit NE40D at 8 % coating level (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 an increase in outer coating level of Eudragit FS30D (table 5). Batch F4 with 12 % coating of Eudragit NE40D could sustain drug release up to 18 h. However, for efficient colon targeting, minimum drug release in initial 2 h and almost complete release up to 24 h is desirable [39] (Dukic-Ott *et al.*, 2008). Hence, results indicated inappropriateness of coating levels in F1-F4 batches. Batches F5 and F6 showed 5-ASA 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 NE40D (16 %) in batches F7, F8 and F9 were responsible for retardation of drug release. Accordingly, the foci for getting optimum coating levels were centered on F5 and F6 batches which showed 98.42±1.23 % and 91.88±0.91 % of 5-ASA 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-ASA release in 2 h, demonstrating the gastroresistancy of Eudragit FS30D at 12 % and 16 % coating levels. Nevertheless, batch F5 is proposed superior over F6 (both with 12 % Eudragit NE40D level) since not only did batch F5 showed nearly complete drug release in 24 h along with comparable release in initial 2 h as F6, but also the cost of the polymer (that can have a significant role for large scale production of pellets) can be considered as an important selection criterion. Additionally, batch F5 showed only 27.85±0.41 % 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 [27].

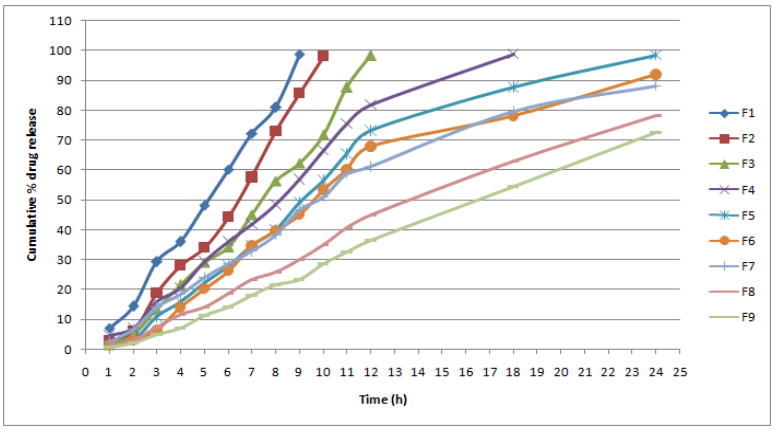


Fig. 4: *In vitro* percent cumulative drug release graph of formulations F1 to F9 containing 5-ASA in pH 1.2 (2 h), in pH 7.4 (2-5 h) and in pH 6.8 (5-24 h) maintained at 37±0.5 °C (mean±SD, n = 3)

The rate of drug release was decreased with an increase in the inner coating level as Eudragit NE40D is water-insoluble and diffusion-release polymer over the entire pH range. It was also realized that higher coating level of Eudragit FS30D created larger lag time compared to the lower coating levels. Accordingly, the optimum formulation was selected based on the feasibility and cost-effectiveness of manufacturing, and lag time of 6-8 h (resembling the time when drug formulations have adequately been localized in the colon).

***In vivo* study**

5-Aminosalicylic acid plasma levels

5-ASA plasma concentrations (µg/ml) obtained after oral administration of uncoated pellets and coated pellets, post-administration a dose shown in fig 5. Maximum peak plasma concentrations of 5-ASA were reached faster after oral administration of the uncoated formulation (mean C_{max} of 1205.28±0.37 µg/ml at 2 h after administration) and in case of coated formulation (mean C_{max} 465.94±0.21 µg/ml and t_{max} of 8 h). The mean $AUC_{0\text{-}24h}$ values obtained with the uncoated and coated formulations were 4325.58 and 5870.21 µg h/ml, respectively.

The drug was released from the uncoated pellets without any delay i.e. showed high plasma concentrations in just 2 h, whereas for the coated formulation, drug release was much lower and only increased after 8 h of administration. The difference *in vivo* behavior of both formulations suggests the efficiency of coating.

Mouth to ileum transit times of cell wall material in rabbits was found to vary between 6 to 7.9 h [40]. In another study, 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) [41]. Following administration of the uncoated formulation and based on the previously documented transit times, 5-ASA release and absorption should have occurred mainly in the rabbit's stomach and proximal small intestine. On the other hand, following administration of the coated formulation, 5-ASA release and subsequent absorption were more significant upon entering the rabbit's caecum. In the literature, the upper intestines are reported to be the preferential site within the gastrointestinal tract for 5-ASA absorption. For example, in humans, 5-ASA absorption through the duodenum was reported to be 5-fold higher than in the ileum [42]. Therefore, the finding that the plasma level of 5-ASA after administration of the coated formulation remained low for 8 h (i.e. during the transit of the pellets through the stomach and small intestine) indicates that the coating was able to prevent drug release at these sites.

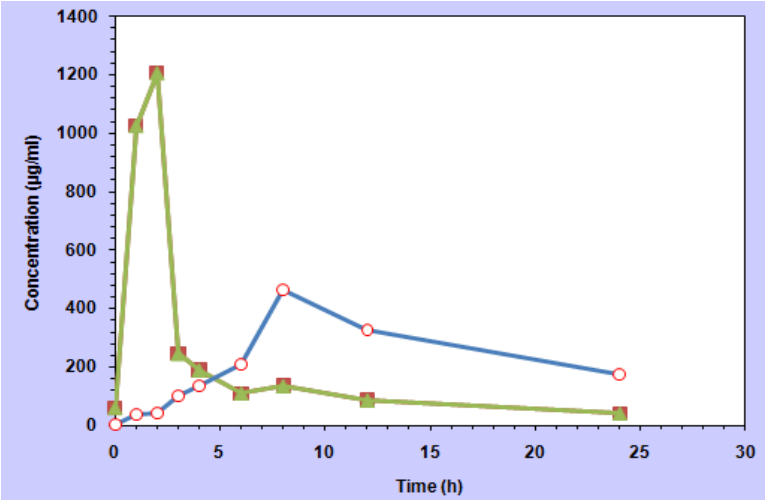


Fig. 5: 5-Aminosalicylic acid Plasma concentration vs Time curve obtained for uncoated and coated pellets (mean±SD)

CONCLUSION

Overall, the study proposes a method for the preparation of 5-ASA loaded pellets for colon-specific delivery with prominent *in vitro-in vivo* correlation and with scalable-industrially acceptable manufacturing process. Extrusion-spheronization was found to be an efficient pelletization method for achieving sphericity and strength. Additionally, this fast and efficient technique offers high drug loading capacity. The combination of pH-sensitive and time-dependent polymers in development of pellets provides assurance of effective colon targeting of 5-ASA. The aqueous coating and Wurster process further strengthen our efforts in development of and promising approach to achieve lag time of 8 h considering the bioavailability of the molecule.

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.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

The authors of this scientific publication report no conflict of interest in this work.

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