Curcumin loaded polysaccharide based micro particles for ulcerative colitis: Preparation, characterization, *in vitro/in vivo* evaluation

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ABSTRACT

The unique pharmacological and therapeutic applications of targeted drug delivery based research have gained its popularity day by day. Targeted therapy delivers the drug at pathological site, increases the bioavailability and enhances therapeutic effect. Curcumin is a natural polyphenol molecule derived from the Curcuma longa used for the treatment of various diseases. It has poor bioavailability and suboptimal pharmacokinetics. To improve its applicability in the colonic diseases, we encapsulated curcumin by multiparticulate coating with inner guar gum and outer Eudragit FS30D. Curcumin loaded multiparticulate based formulations were prepared and characterized for particle morphology, particle size, drug encapsulation, equilibrium swelling studies, in-vitro drug release, stability study and in-vivo histopathological study for the treatment of ulcerative colitis. Our result showed that particles were of spherical shape with an average size range of 500 µm with 72.5% encapsulation efficiency. The drug released was found to be 92.25% in rat caecal medium. The in-vitro release study demonstrates that encapsulation of curcumin by multiparticulate system was successful in manipulation of human gastrointestinal tract physiology. Results of the histopathological study, clearly proves that encapsulation of curcumin was found to be the best therapeutic option for the treatment of ulcerative colitis.

Key words: Colon-targeted drug delivery system, multiparticulate system, pH and microbial approach, curcumin, guar gum, and Eudragit FS 30D, Ulcerative colitis.
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INTRODUCTION

Over the years, due to lack of understanding or appreciation of the human colon was regarded as a needless organ which absorbs water and electrolytes. It is considered as a suitable site for the safe and slow absorption of drugs which are targeted at the large intestine, designed to act systemically [1]. Site specific delivery of drugs to the colon provides major advantages to the diseases associated with colon [2] and protein drug delivery [3]. Colon targeted drug delivery is expected to reduce their side effects and to increase the pharmacologic response. For controlled release system, oral route of administration is the first choice because the physiology of our gastrointestinal tract (GIT) allows us to design more varied dosage forms as compared to the other routes [4]. Moreover, oral route is the most versatile and commonly employed route for systemic action, due to its ease of administration, patient compliance and flexibility in formulation. Therefore, it appears that oral colon targeted dosage forms with an appropriate release pattern could be very useful in providing effective therapy for colonic diseases [5].
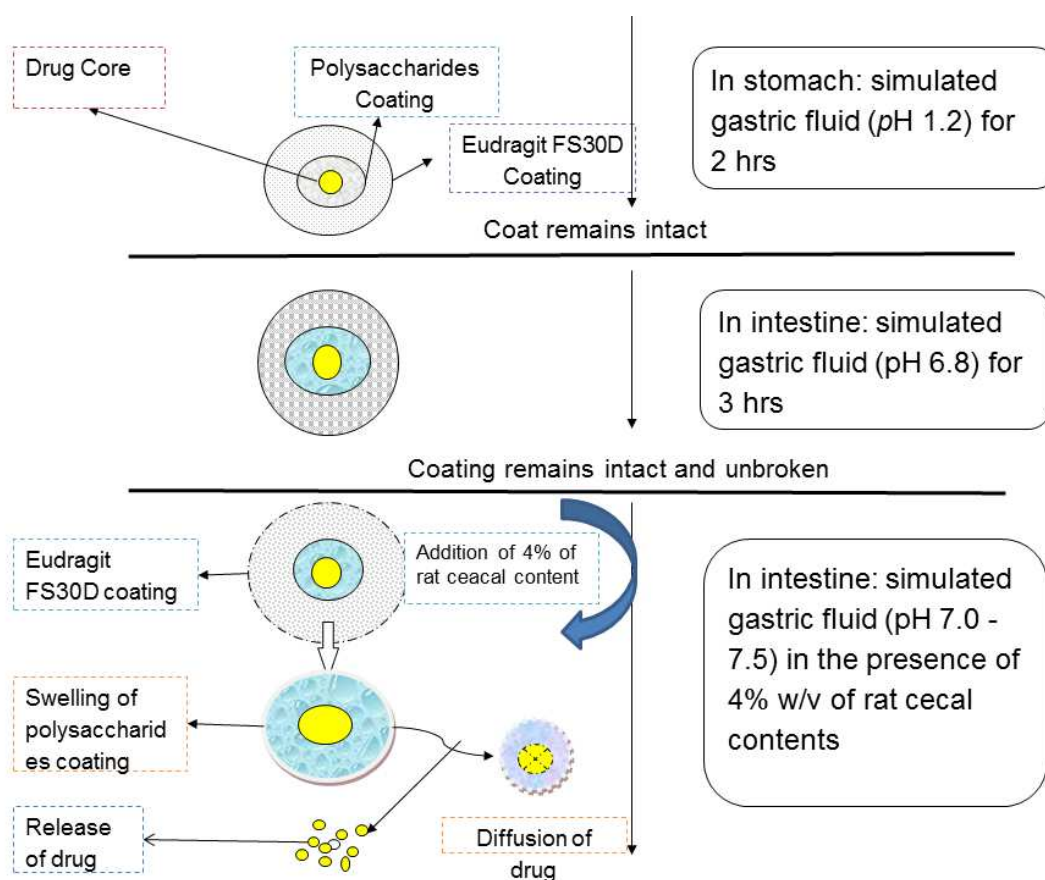
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Figure 1. Schematic representation of drug release from Curcumin loaded granules using Eudragit FS30D and guar-gum in colon

Although, colon targeted drug delivery is advantageous in the treatment of various diseases i.e colorectal cancer, Crohn's disease, ulcerative colitis, irritable bowel syndrome, and spastic colon [6]. But drug delivery to the colon by oral route is still a challenge for the researcher due to its location at the distal portion of the alimentary canal and is, therefore, difficult to access [7]. And in GIT, different enzymes and a wide range of pH values are present (pH 1.2-8), when dosage form is given, it has to travel through these pH range and enzymes, before reaching the target site, further complicating the reliability and delivery efficiency [8]. There is misconception among the investigators that they have understood the physiology of our GIT. But, the reality is that the physiology of GIT is underestimated [9, 10]. To knock off from this misconception, there is a need to understand the pathophysiological conditions in the GIT under diseased conditions. Pathophysiological conditions of a healthy person and patient suffering from colonic diseases might significantly differ from those in the physiological state [11]. For instance, different types of bacteria (e.g., bacteroides and bifidobacteria) are generally present in the healthy colon and able to degrade complex polysaccharides due to multiple extracellular glycosidases [12, 13]. However, in the disease state their concentrations of colonic bacteria can be significantly reduced [14, 15]. To overcome these problems, there is a need to make a strong bridge between the mechanisms of action and exact GIT physiology in diseased conditions. These misconceptions have been addressed by investigator by using various targeting approaches of drug delivery systems as described in the literature in order to provide such targeted drug delivery to the colon i.e. time-based colonic drug delivery [16-18], pH dependent colonic drug delivery [19-22], prodrugs approach for colonic drug delivery [23,24], polysaccharides based colonic drug delivery [25-27].

The study aims to find out the effect of the colonic delivery of curcumin on the subsequent doses of the formulation, wherein combination of xanthan gum and guar gum have been used for targeting. Aim is to give a solution to the problem of premature colonic release of the drug in the treatment of ulcerative colitis. It was envisaged to overcome the problem by the dual-coating of curcumin. Due to their potential therapeutic benefits, increased bioavailability and decreased side-effects multiparticulate based system were chosen for the formulation development [42-44]. Mixture of guar gum with xanthan gum is used in a curcumin based core development and coating with the solution

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of guar-gum, determining its potential use in colon specific drug delivery systems coupled with an outer Eudragit FS 30D coating. Particular aims included: Inner gum coating and outer with Eudragit FS 30D consecutively onto dry granules (**Figure 1**). The proposed approach will improve targeting the drug to the affected site and polysaccharide degradation by colonic bacteria responsible for the release of the drug from the coated tablets.

EXPERIMENTAL SECTION

The active material (Curcumin) was purchased from K. Patel Phyto Extractions Pvt. Ltd, Mumbai, India. Guar gum having a viscosity of 5200-5500 cps and Xanthan gum were obtained from Molychem Manufacturers (P) Ltd, Mumbai. Eudragit FS 30D was an Evonik Industries (Germany) product.

Preparation of drug loaded core granules
The granules of curcumin containing equal composition of drug to polymer ratio were prepared by a dry granulation method. The composition of curcumin, guar gum and xanthan gum were at a ratio of 1:1:1 (15 gm each), respectively. Polymers were passed separately through sieves no. 80 and were mixed with curcumin. The ingredients were mixed thoroughly in a V-cone blender.

Inner guar gum coating of the prepared granules
The prepared granules were coated up to 20% with a guar gum dispersion of 4% w/w in ethanol/water (20% v/v) in an accela cota coating pan to a definite cumulative coating weight gain. Cumulative coating weight was calculated by,

(Final weight – Original weight)/ Original weight X 100

After coating, the granules were dried for 5 h at 65^0 C. The inner coating with guar gum offers an additional protection to the multi particulate unit, until it is degraded by microbial enzymes at the proximal colon.

Outer Eudragit FS30D coating of Guar-gum coated granules
Further, the guar gum coated granules were again coated with 40% of Eudragit FS 30D in an accela cota coating pan. Outer coating with Eudragit FS30D, works as a time-controlled retardant and offers additional protection to the drug released from its dosage form. In an introductory study, a 15 to 20 % coating was capable of premature release of the drug at a stomach pH of 1.2. Coating of the granules up to 40% retarded drug release at an intestinal pH of 6.8.

Filling of coated granules in hard gelatin transparent capsules
The weight equivalent to 100mg of coated granules of curcumin accurately filled in hard gelatin capsules in a lab scale capsule filling machine.

Shape and surface morphology
Curcumin loaded multiparticulate were observed under a scanning electron microscope (SEM- Jeol, JSM-6100). Samples were prepared by sprinkling granules powder on a double adhesive tape, which was stuck on an aluminum stub, further it is coated with gold using a sputter coater, and photographs of the samples were taken for shape and surface morphology [45].

Particle size analysis
Particle size was determined using a laser diffraction particle size analyzer, Malvern Mastersizer. The coated granules were suspended in the chamber of the particle size analyzer containing distilled water, and the particle size was determined using the software provided by the manufacturer [46].

Determination of drug content and encapsulation efficiency
Encapsulation efficiency is the amount of added drug encapsulated in the formulation in terms of percentage. Accurately weighed formulated granules (100 mg) were finely powdered and dispersed in a 100 mL volumetric flask containing buffer at a pH of 1.2. The samples were shaken 15 minutes each, with a resting period of 5 minutes each. They were left for 10 hours at room temperature to equilibrate. After reaching the equilibrium, they were centrifuged at 3000 rpm for 10 minutes. The concentration of curcumin in the decanted Tris HCl buffer was determined at 425 nm in a spectrophotometer [47].

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Equilibrium swelling studies
Coated granules (100 mg) were placed in phosphate buffer saline (PBS) at a pH of 7.4 and were allowed to swell up for 24 hrs to a constant weight. The granules were filtered and changes in weight were recorded. The degree of swelling (α) was calculated according to the given formula given below,

α = Final weight – initial weight/ Initial weight x 100

Preparation of fresh rat cecal content medium
Albino rats weighing from 150-200 g in range were selected for the present study. All the experimental procedures were approved by the Institutional Animal Ethics Committee of Department of Sciences and Technology, Birla Institute of Technology vide Approval Number BIT/PH/IAEC/06/2014. The animals were housed in the animal house in clean poly- acrylic cages and maintained under day and night cycles at an average temperature of 25±2°C. They were maintained on a normal diet (Bengal gram purchased from local market and soaked in water).

All the experimental rats were treated with 1 ml of 2% w/v of guar gum dispersion for assessing the susceptibility of guar gum to colonic bacterial degradation. The procedure of oral treatment was carried out for 7 days. Thirty minutes before the commencement of the drug released studies, six rats were euthanized. The abdomen was opened and cecum contents isolated, legated at both of the ends, dissected and transferred immediately to a pH of 7.4 PBS, previously bubbled with nitrogen. A bag containing cecum was opened, contents were weighed individually, pooled and suspended in the buffer continuously bubbled with nitrogen to give a 4 % w/v dilution. All the above processes were carried out under nitrogen in order to maintain anaerobic conditions.

***In-vitro* drug release of guar gum and Eudragit FS 30D coated granules of curcumin using rat caecal contents**
In-vitro drug release study was carried out in USP I (basket type) dissolution test apparatus in the presence of rat caecal contents. However, a slight modification in the procedure was done. The experiments were carried out in a 250 ml beaker immersed in water maintained in the jars of the dissolution test apparatus. Six capsules of curcumin were subjected to each of the vessels (beakers) containing the dissolution medium. Dissolution study of curcumin capsules was carried out in 150 ml of pH 1.2 HCl buffer at 50 rpm at 37 ± 0.5°C for 2 hours. Afterwards, the pH of the dissolution media was adjusted to 6.8 using 50 ml of pH 6.8 phosphate buffer saline and the study was continued for up to 5 h. At the end of the fifth hour, the media was degassed using nitrogen gas to remove the undissolved oxygen to maintain anaerobic conditions inside the medium for 15 mins. 4% w/v of freshly prepared rat cecal solution was added to the dissolution media and the study continued for up to 24 h under the continuous purging of nitrogen throughout the study. About 5 ml of samples were withdrawn at the time interval ranging from 0.5-24.0 h), respectively, from the dissolution medium and were replaced by the fresh medium which was maintained under anaerobic condition. All the studies were repeated six times and the mean data was recorded.

Stability of dual-coated granules
Stability studies were carried out according to ICH guidelines. In this study, coated granules were sealed in aluminum packaging coated inside with polyethylene, and three replicates were kept in a humidity chamber maintained at 40 ± 2° C and 75 ± 5% RH for three months[48] Samples were collected after three months of storage and analyzed for drug content and *in vitro* dissolution rate[49]. After successful completion of 90 days, the sample were subjected to dissolution studies as per the method described above to verify whether any changes in dissolution profiles took place due to stability issues.

***In-vivo* evaluation of multiparticulate coated granules of curcumin**
Experimental Design - **Induction of colitis and Treatment Schedule**
Ulcerative colitis was induced by the use of dilute acetic acid. Rats were anesthetized with a dose of 75 mg/kg ketamine injected intraperitoneally. A flexible plastic catheter with an outer diameter of 2 mm was then inserted rectally into the colon with the aim to place the catheter tip 8 cm proximal to the anus. Colitis was induced by intracolonic instillation of 2 ml/day 2 % v/v acetic acid with 24-hrs interval for three constitutive days. Injected rats were maintained in a head-down position for 2 mins to prevent solution leakage.

The animals were randomly divided into three groups each of six animals. After induction of ulcerative colitis in rats, they were subjected to 17 days of dosing period. The dosages were freshly prepared every day by suspending the drug/ formulation in water and administered by oral gavage needle once daily. The distribution of groups and dosage schedule are given in (**Table 1**).

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Table 1 Distribution of animals with dosage schedule

Groups	No. of Animals	Treatment	Dose (p.o)
Group-I	6	Normal control	50 ml/kg
Group-II	6	2 %v/v Acetic acid	2 ml/kg (intracolonic)
Group-III	6	Curcumin-powder + 2 %v/v Acetic acid	500mg/kg+2ml/kg
Group IV	6	Formulation of Curcumin +2 %v/v Acetic acid	500mg/kg + 2ml/kg

Histological evaluation

Animals were sacrificed at the end of the 17 days treatment and colonic tissue samples were taken and fixed in freshly prepared 10% formalin solution. The samples were examined histopathologically to study the anti-ulcerogenic effect of various formulations used for the treatment of UC as mentioned in table 1. The transverse sections of colonic tissues were taken and embedded in paraffin blocks. These sections were stained with hematoxylin and eosin. The slides were examined microscopically for patho-morphological changes such as congestion, hemorrhage, inflammation, erosion and ulceration in the mucosal, submucosal, muscular and serosal layers of colon specimen.

RESULTS AND DISCUSSION

Granules characteristics and drug content efficiency

The film coating with guar gum aqueous dispersions was very difficult because of its high viscosity nature. A low concentration of 1%w/v of guar dispersion sprayed with spray gun in accela cota was used. Drug content and encapsulation efficiency are important parameters to evaluate the drug content of multiparticulate granules. Drug content of the GG/Eudragit FS 30D coated granules of curcumin was found to be 72.5%.

The average particle size of the coated granules was found to be 500 µm (**Figure 2**). As the main objective of this study was to investigate the effect of guar gum and Eudragit FS 30D coating, the drug layer was designed with instant and prolonged release characteristics.

In-vitro **drug release study**

The core of the granule containing curcumin was spray-coated in an accela cota pan coater with guar gum and Eudragit FS 30D. In the preliminary studies, more than 80% of the drug was released within 1 hour. This type of burst release is not accepted for drugs that are required to be released at a specific site or locally to the colon.

In-vitro drug release profiles of coated granules with guar gum showed a biphasic release with an initial time based delay due to highest viscosity of gum. As the coating level is higher, drug released profile is slower as compared to uncoated granules. As the viscosity of the guar gum increased, when it came in contact with water, it started swelling and a gel like film was formed. This gel like film acted as a diffusion barrier around the core. Once water penetrates, a porous structure was formed and curcumin began to diffuse outside the protective barrier. The drug release was found to be 35 % within 1 hour.

In the third part of dissolution investigation, guar gum coated curcumin was coated with a pH sensitive coating polymer Eudragit FS 30D for the successful delivery of curcumin to the colon. It is mandatory to mimic the entire pH of the GIT to overcome the premature and precolonic release of drugs from its formulation. In the structure of improved colon targeted drug delivery, the inner coating of granules with guar gum will act as time release drug delivery and the Eudragit FS 30D coating will act as a pH dependent drug delivery system. There was no drug released in the first 3 hours of this study. Referring to the double coating of granules with guar gum and the Eudragit FS 30D drug releases, experiments were carried out for 24 hours in an anaerobic media containing 4 % w/v of rat caecal contents. The *in-vitro* drug release study of the multiparticulate coating curcumin granules showed that only 3% of the drug was released in simulated gastric and intestinal fluids. After 5 h, cecum contents were introduced and the cumulative percent drug release was found to be 17.5 %. The total amount of curcumin released from coated granules was found to be 92.25% after 24 h (**Figure 3**).
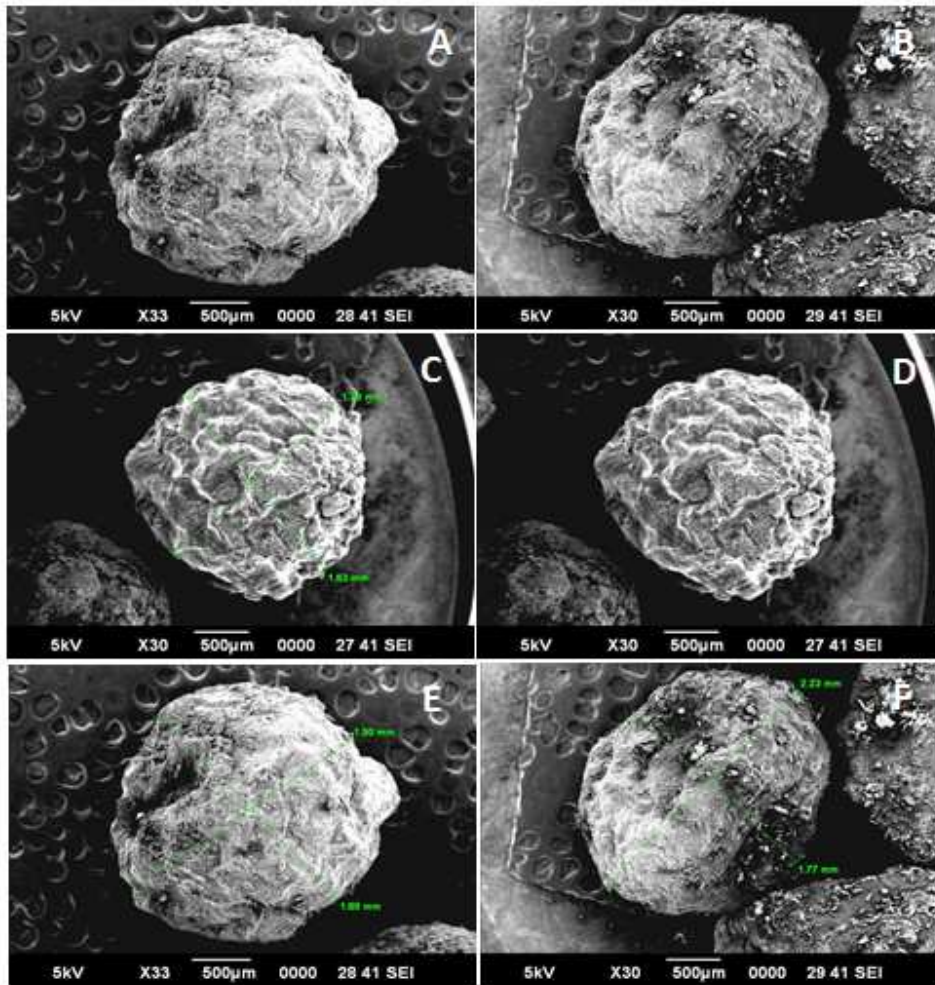
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Figure 2. Different Scanning electron microscopy (SEM) of multiparticulate particles of Curcumin coated with guar gum and Eudragit FS30D (1:1:1)
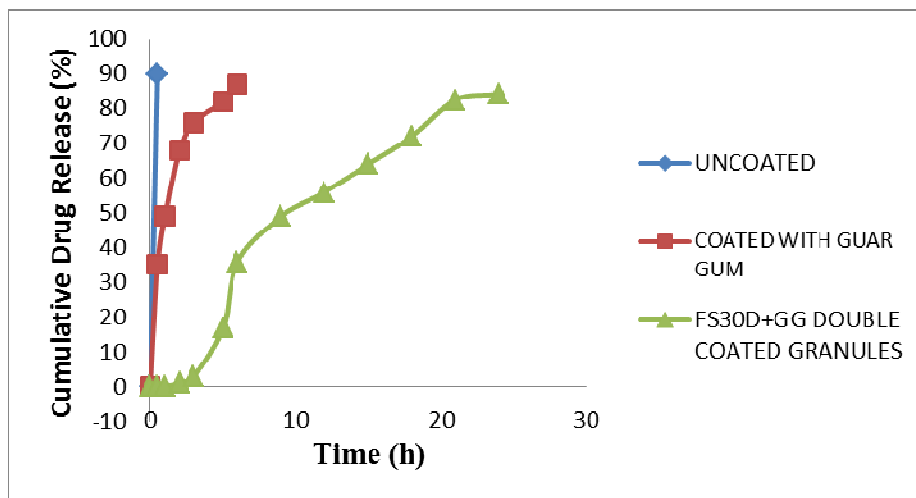

Figure 3. *In-vitro* drug release study of Curcumin loaded granules using Eudragit FS30D and guar-gum

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In the pH dependent systems, polymers are based on the difference in pH levels along the GIT. The poor site specificity of pH dependent systems due to a large variation in the entire pH of the GIT has been very well established [28, 29]. Although, there are various delivery systems present, but microflora activated delivery systems have been found to be the most promising. The rapid increase of the bacteria population and associated enzymatic activities in the ascending colon represents a non-continuous event independent of GI transit time and pH [30, 31]. Therefore, in light of premature and pre- colonic spontaneous release of drug from its dosage form, here, our main concern was to developed safest and effective colon targeted drug delivery of curcumin.

Researchers reported that polysaccharides from the natural source (such as xanthan gum, guar gum, chitosan, pectins, etc.) showed synergic effects for colonic drug delivery [32]. These polysaccharides are not affected by acidic pH of stomach and alkaline pH of small intestine. Degradation only starts in the presence of the vast an aerobic microflora of the colon (for example, bacteroides, bifidobacteria, and eubacteria) to smaller monosaccharides [33]. On the basis, of polysaccharides based drug delivery system which is non-digestible and fibrous in nature, act as prebiotics. These prebiotics carrier can only degrade in the presence of gastrointestinal microflora. Most of the polysaccharides based carrier systems are either in matrix or compression coated tablet form [34, 35]. But, the best thing about these systems is slow and controlled release of drug from its dosage form. By understanding of the intestinal environment we developed our rationale. In the present investigation a novel multiparticulate colon-specific drug delivery of curcumin has been developed by inner coating with guar gum. The natural gums coating (guar gum) play an important role i.e. in intestinal conditions to allow its release only in the colon and also serve as prebiotics to provide nutrition to the colonic bacteria that are responsible for the removal of the protective covering formed by the gums. Outer coat of granules with Eudragit FS 30D, are stable in gastric pH and releases in ileum or colon ascendant starting at 7 pH. Dynamic nature of Eudragit FS 30D gives protection to the drug and allow for the site-specific delivery of drugs to the colon [36-41].

The dual-coating multiparticulate system was based on the presumption that targeted release would occur in the colon because colonic micro flora would assist in digesting 'polymeric coating' of the curcumin, and the outer Eudragit FS30D coating results in release of the drug at particular colonic pH. It was presumed that colonic micro flora would not be affected by the drug released in the colon and only diseased would be affected. This was a cause of concern to inventors. Therefore, for an effective and targeted drug delivery of the drug via oral route, an innovative system is disclosed.

Stability studies
Stability studies of the multiparticulate granules were carried out at 40 ±2° C and 75 ± 5% RH for three months to assess their potential utility. After storage for three months, the granules were subjected to drug content and *in-vitro* dissolution studies. Results of the stability study showed that there was no marked difference in drug content and dissolution profiles of granules before and after storage.

Histological examination
Light micrograph of middle colon suggested that the pathological changes were seen among the different groups by different treatments given. Microscopy study suggested that the colon shows intact mucosa lined by glandular epithelium [Fig.4 (A), Long Arrow] and the muscular layer [Fig.4 (A), Short arrow] and serosal layer appear unremarkable. Diffuse ulceration of the colonic mucosa with loss of lining epithelium was observed in case of 2% W/V acetic acid treated rats [Fig.4 (B), Long Arrow]. The submucosal layer shows moderate edema with inflammatory infiltration [Fig.4 (B), Short Arrow]. The muscular and serosal layers appear unremarkable. Section studied shows intact colonic mucosa with intact lining epithelium [Long Arrow, Fig.4 (C)]. The submucosal layer [Short Arrow, Fig.4 (C)], muscular and serosal layers appear within normal limits. Section studied shows intact intestinal mucosa (Long Arrow, Fig. 4 (D)). Intervening the glands in the mucosal layer, there are seen mild infiltration by inflammatory cells predominantly comprising of lymphocytes, plasma cells, and macrophages. The submucosal layer shows moderate edema (Short Arrow, Fig.4 (D)). The muscular and serosal layers appear unremarkable.
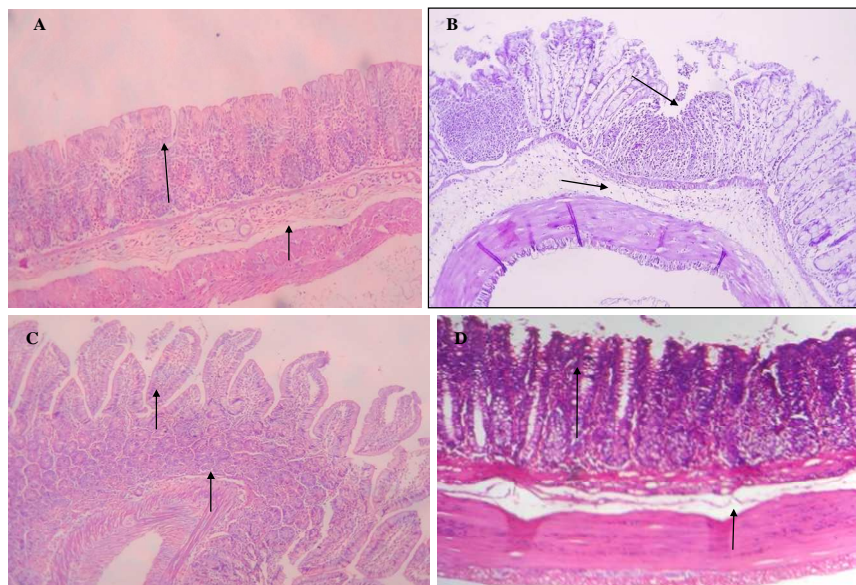
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Figure 4. Histological view of transverse section of colonic specimen prepared and stained with Hematoxylin–eosin stain as seen under light microscope at 50 x magnification (A) Normal control (B) 2 %v/v Acetic acid (C) Curcumin-powder + 2 %v/v Acetic acid (D) Formulation of Curcumin +2 %v/v Acetic acid

CONCLUSION

In this present study, novel multi-particulate based formulation system was developed for the colon targeted drug delivery system. In this system, drug release profiles were controlled by using both natural as well as synthetic polymer. The double fold coating approach of granules were aimed to target drug delivery to the inflamed colon while preventing the burst release of drugs in the stomach and small intestine. Multiparticulate colonic drug delivery was developed and tested _in-vitro_ simulating the colonic condition by using cecal content medium. Film coating with guar gum was successfully performed using an aqueous dispersion of guar gum. Furthermore, under gradient pH conditions (pH=1.2 for 2 h; pH=6.8 for 5 h; pH=7.4 for 24 h), it was observed that after changing the pH of the medium to 6.8 and adding rat cecal contents, drug release was accelerated. The coated multiparticulate system showed release of the drug specifically at the target site after suitable lag time and may contribute in the effective treatment of colonic diseases, which needs to be further proven by _in-vivo_ experiments. The outcomes of the study showed that it was successful in minimizing the release of the drug off target area (pH 1.2 and 6.8) and to maximize the release of the drug in target area (pH 7.4), which is the primary requirement of colon specific targeted drug delivery. _In-vitro_ drug released study of pH and microflora activated colon-targeted drug delivery systems of curcumin gave a good indication and high degree of protection and prevention of drugs from its dosage forms. Outcomes of the _in-vivo_ results have shown that encapsulation of curcumin by multiparticulate coating were successful in the treatment of ulcerative colitis without premature release of the drug. In conclusion, the development of a multiparticulate based colon targeted drug delivery method is a good approach for colon targeting of various drugs. Multiparticulate based formulation may be further helpful for the pre-clinical and clinical trial study.

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