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Formulation Development and Evaluation of Colon Targeted Beads of Mesalamine

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Abstract

The aim to develop a locally effective colon targeted therapeutic system in the form of a multiparticulate oral formulation that might provide an improved efficacy with a significant reduction in dose and systemic toxicity and improved quality of life for the treatment of Irritable Bowel Disease (IBD). The formulation of beads develops by inotropic gelation. The effect of various formulation parameters such as ratio of gellan gum and locust bean gum were studied for drug loading, and entrapment efficiency. To retard the drug release in the stomach and achieve a pH dependent release at colonic pH of 7.4 a polymethacrylate base coating carried out by dip coating method. The surface analysis was carried out by scanning electron microscopy (SEM). In vitro analysis of coated formulation shows much retarded release in comparison of uncoated formulation. The in vivo release performance of the developed colon specific formulation was carried out by gammascintigraphy.

INTRODUCTION

Dosage forms that deliver drugs into the colon rather than upper gastrointestinal tract (GIT) offers number of advantages [1]. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, Crohn's disease, carcinomas and infections) whereby high local concentration can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT or unnecessary systemic absorption. Mesalamine is an anti-inflammatory drug used to treat inflammation of the digestive tract ulcerative colitis and mild-to-moderate Crohn's disease. Mesalazine is a bowel-specific aminosalicylate drug that acts locally in the gut and has its predominant actions there, thereby having few systemic side effects [2-4]. As a derivative of salicylic acid, 5-ASA is also thought to be an antioxidant that traps free radicals, which are potentially damaging by products of metabolism. The empirical formula is $C_7H_7NO_3$, representing a molecular weight is 153.14 g/Mol, It is white to pinkish crystals slightly soluble in water, 20 to 30% absorbed followed oral administrations [5].

Gellan gum, are natural biodegradable and biocompatible gums shows a significant promise for colon targeting of drug. The locust bean gum a hydrophilic polymer which is used to prolong release of drug from the formulation. Due to ionic gelation process that affects the entrapment efficiency of the drug. This limitation is overcome by the use of mixture of gum in which one gum has improved ionic gelation property such as gellan gum, sodium alginate etc. Several investigators proved, work on mixture of the natural polysaccharide in different proportion for the development of colon targeted drug delivery system. The various approaches was used for development of the colon targeted system includes coated formulation with pH sensitive copolymer and with the release of the drug started in alkaline pH of intestine. This was the most common approaches which was used by several investigators in past and other approach also suggested that use of biodegradable polymer's coating which degrades by the action of the colonic bacteria and release of drug at the colon. Another method for colon targeted drug delivery was develop the pH sensitive matrix in which the drug release started with the degradation of pH sensitive polymer in the GIT [5,6]. Also other two approaches out of which one was bioadhesive system and other prolonged time released systems. When the formulation coated with bioadhesive polymer that selectively provides adhesion to the colonic mucosa, on the other hand formulation was designed such a manner that the drug releases should after a lag time of 3-5 hrs that is equivalent to small intestine transit time [7].

The precise mechanism of action of mesalamine is not known, but is likely due to a combination of anti-inflammatory properties. Mesalamine has been shown to block the production

of interleukin-1 (IL-1) and tumour necrosis factor-a (TNF-a) [8-10]. Sulfasalazine has also been found to inhibit binding of TNF-a to its receptor, thereby preventing signalling of subsequent inflammatory responses [11]. Mesalamine is a potent inhibitor of the cyclooxygenase pathway, inhibiting the production of prostaglandin E2 in inflamed intestinal specimens [12,13]. Blockage of the lipooxygenase pathway also has been shown [14,15] inhibiting both 5-lipooxygenase and 5-lipooxygenase-activating protein, which in-turn blocks the production and chemotactic activity of leukotrienes such as leukotriene B4 (LTB4) and hydroxyeicosatetraenoic acid (5-HETE)). Its efficacy as an anti-inflammatory agent is also thought to be due to effects on leukotrienes metabolism [16,17]. Mesalamine is also one of the most potent known free radical scavengers and antioxidants [18,19].

MATERIAL AND METHODS

Mesalamine and Eudragit S-100 was procure as a gift sample from the Wallace Pharmaceuticals Pvt Ltd, Goa and Evonik Roehm Pharma Ltd., Mumbai , Locust bean gum was purchased from the Himedia Laboratories Pvt. Ltd, Mumbai. Gellan gum and sodium alginate was purchased from SRL chemicals Pvt. Ltd, Mumbai, S.D. Fine Chemical Ltd, Mumbai respectively. Pepsin, Pancreatin and Galactomanase was purchased from Himedia Laboratories and Sigma Aldrich, Mumbai respectively.

Preparation of mesalamine beads

For encapsulation of drug in gellan-locust-alginate beads, physical mixtures of gellan gum locust bean gum and sodium alginate in ratio 1:3:2, 1:1:2 and 3:1:2 were dispersed in 40 ml of 40°C double distilled water by magnetic stirring. Mesalamine (750mg) was suspended in gel mixture (1:1 ratio with physical mixture) and vigorously stirred for 1hr. Then using a No. 20 hypodermic needle, drug-gel solution was added drop wise into 1M $CaCl_2$ solution. The solution was continuously stirred with help of magnetic stirrer for half an hour; beads were filtered from the solution and kept for drying [6] (Table 1).

Coating of beads

The beads were coated with 10% Eudragit S100 solution in chloroform: methanol mixture (72:28 v/v) by typical solvent evaporation method. Dip Coating was followed to develop the beads. The beads was alternatively dipped in 10% Eudragit S-100 solution and dried. After drying, the weight of beads was measured and the coating was ruled with an accurate weight again of 8-12% (Table 1).

Particle size analysis

About 25 mesalamine beads were randomly picked up thrice and their size was measured by using calibrated vernier callipers.

Surface morphology

Surface morphology of the specimens were determined by using a SEM, Hitachi model SU 1500. The dried samples were mounted on brass specimen studies, using double sided adhesive tape. Gold-palladium alloy of 1200A knees was coated on the sample using sputter coating unit (JEOL JFC-Model 1100E, Japan) in Argon at ambient of 8-10 Pascal with plasma voltage about 20 MA. The sputtering was done for nearly 5 min.

Drug content determination

Fifty mg of uncoated sample is first extracted with 10ml of SCF by continuously stirring for at least 1 hour in a glass jar. Then 10 ml of simulated colonic fluid (pH 7.4) with little quantity of gamanase enzyme add into the glass jar. Pipette out 1ml of this solution in 100ml volumetric flask and diluted with simulated colonic fluid and measure the absorbance in UV- Visible Spectrophotometer. Drug entrapment efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula % Entrapment Efficiency = 100 % Mass of Drug Used in Formulations

In vitro release studies

The *in vitro* release studies of prepared beads were carried out in simulated gastric fluid (pH 1.2) for 2hrs, simulated intestinal fluid (pH 6.5) for 3 to 5 hrs and simulated colonic fluid (pH 7.4). For 6 to 24 using USP XXIII apparatus at 100 rpm maintained at a temperature of 37+1°C for a period of 24hrs. At periodic time intervals 10ml of sample was withdrawn suitably diluted and absorbance was measured at 330nm.

In vivo studies

In vivo studies of prepared formulations were carried out by determining the *in vivo* behavior of dosage forms in animals. The Scintigraphy of the optimized formulation (CF-3) coated with radiolabel coating and filled in capsule and activity were perform on rabbits in order to establish its colon targeting potential.

Stability study

The optimized formulation (CF-3) was packed in aluminium foil. It was than stored at 40°C / 75 % RH according to ICH guidelines. Samples were withdrawn after three month and evaluated for change in drug release pattern.

RESULTS AND DISCUSSION

Preparation of mesalamine beads

When the drug and gel mixture was add in to the 1M $CaCl_2$ solution due to presence of gellan gum and sodium alginate the spherical shape beads are from which appears a dark colour after the drying. These dry beads were coated with the 10% Eudragit S-100 polymer by the dip coating method.

Particle size

The size of the beads was measured by simple vernier calliper method. The particle sizes of all formulations are shown in Table 2. The particle size of Mesalamine beads was found to be 1.34 mm, 1.28 mm and 1.26 mm for the uncoated beads of formulation F1 to F3. When these beads coated with the Eudragit S100 by dip coating method the diameter of beads was increase. The size of the beads after coating was found to be 1.56, 1.49 and 1.50mm of three formulations CF1, CF2 & CF3 respectively.

Surface morphology

Scanning Electron Microscopy was used to observe the surface structure of the gellan, locust bean gum and sodium alginate bead before and after coating The SEM analysis revealed that the beads prepared in this study were mostly spheres with rough surfaces. Figure 1 shows the appearance of the white spots on the surface of alginate/gellan gum/locust bean gum beads. The size of spherical beads ranged from 1.26 to 1.50mm (Table 2) (Figure 1).

Drug content and entrapment efficiency

The results of drug content and entrapment efficiency shows

Table 1: Formulation Plan for Mesalamine Beads.

Formulation Code	Ratio of drug and polymer. (Drug: Polymer)	Ratio of Gums Used (GG:LBG)	Ratio of Sodium alginate and gum Used	Coating with 10% w/v eudragit
F1	1:1	1:3	1:2	No
F2	1:1	1:1	1:2	No
F3	1:1	3:1	1:2	No
CF1	1:1	1:3	1:2	Yes
CF2	1:1	1:1	1:2	Yes
CF3	1:1	3:1	1:2	Yes

Table 2: Formula for Mesalamine Bead, Percentage Yield, Percentage Content of Drug and Entrapment Efficiency

Formulation code	Percentage yield (%)	Drug content (%)	Entrapment Efficiency (%)	Particle size (mm)
F1	92.3	39.79	79.57	1.34 ± 0.20
F2	92.4	41.16	82.32	1.28 ± 0.17
F3	93.4	41.54	83.04	1.26 ± 0.22
CF1	92.2	40.54	80.04	1.56 ± 0.19
CF2	92.4	40.16	82.32	1.49 ± 0.15
CF3	93.4	41.54	84.04	1.50 ± 0.22

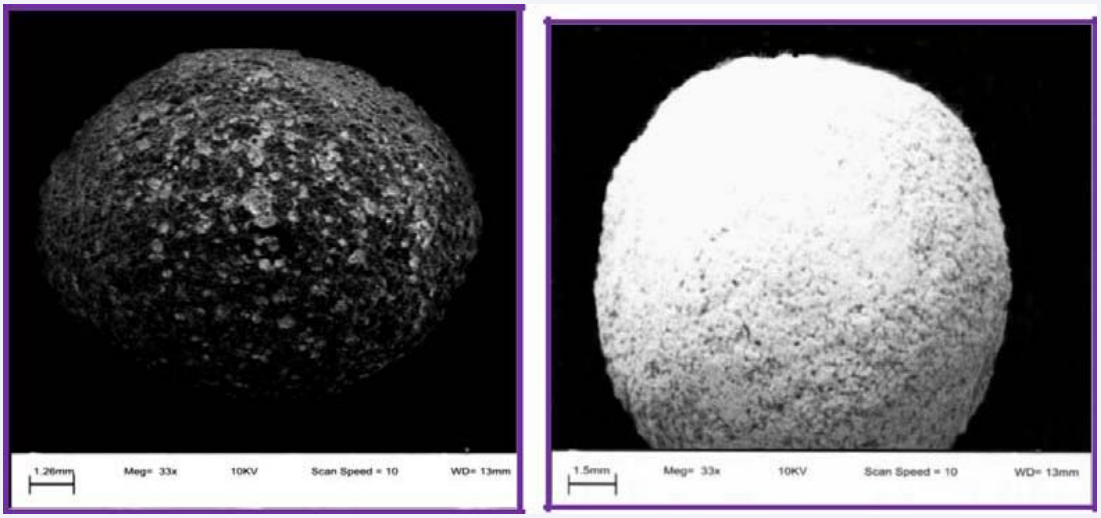

Figure 1 SEM images of (A) Uncoated Bead (B) Coated Beads.

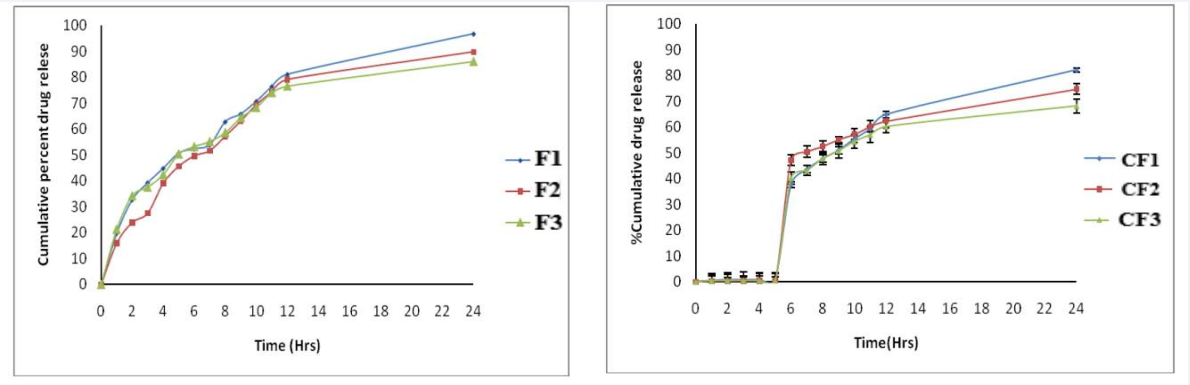

Figure 2 *In vitro* Release Profiles of Mesalamine (Uncoated Formulation F1, F2 &F3) and (Coated Formulation CF1, CF2 & CF3).

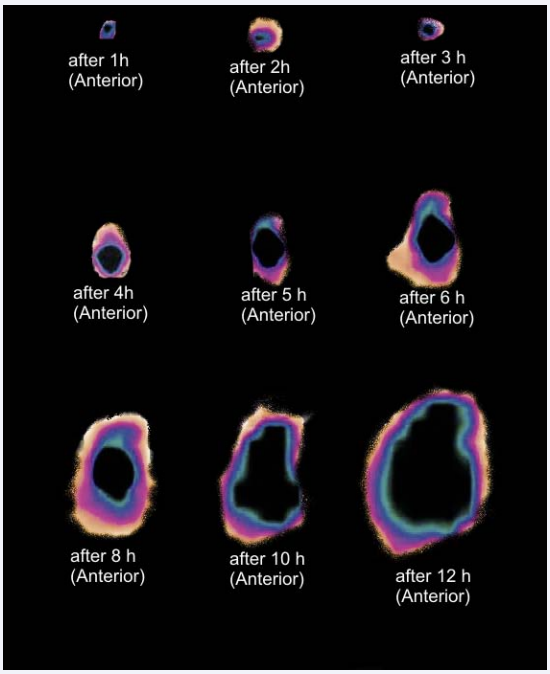

Figure 3 Gamma scintigraphic images of mesalamine loaded gellan beads in rabbit at different time intervals.

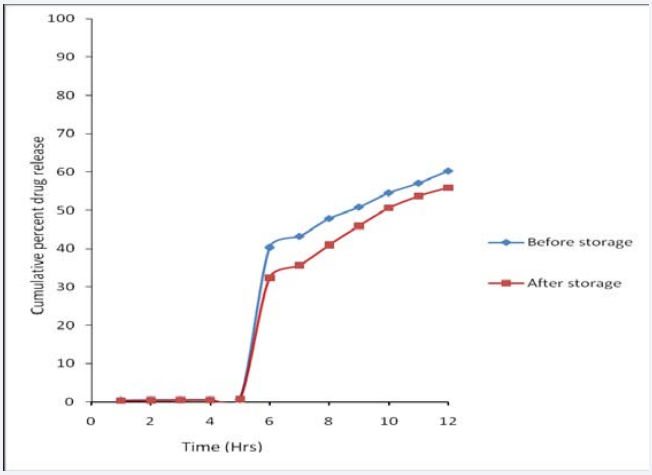

Figure 4 Dissolution profile of Stability Batch.

that both are increase with increase in proportion of gellan gum in the ratio of natural gum mixture this is due to the gellan gum has good in ionic gelation so drug could not come out in the gelation medium (1M $CaCl_2$) [6,12]. The results of drug content and entrapment efficiency are given in Table 2. Due to absence of calcium ions along with poor solubility in distilled water, mesalamine shows the better results in the entrapment efficiency (Table 2).

In vitro drug release

The cumulative percent drug release were studied in three different pH values pH 1.2, pH 6.5 and pH 7.4 which proved the targeting of drug in colon as there is approx. 0% release in pH 1.2 representing the stomach but as the pH increases the release also increases.

The cumulative percent drug release after 24 hrs was found to 96.73%; 89.76%; 86.12% and 82.12%, 74.79%, 68.24% for F-1, F-2 ,F-3 of uncoated and CF1, CF2, CF3 of coated formulation respectively. From the results it was observed that, the uncoated beads release the drug more than Eudragit S-100 coated beads in first five hrs. The results shows that the drug releases for uncoated beads take place more than 50% of drug in the area of upper intestine due to an initial burst effect. This burst effect is consistent with earlier findings of burst effect is due to poorly entrapped drug on the surface of gellan gum beads. By comparing the *in vitro* release pattern of all coated and uncoated formulations, it was found that the drug release from coated formulations was prolonged than uncoated beads. The drug release may be mainly controlled by drug diffusion through the natural gums matrix (Figure 2).

In vivo studies

In order to investigate out the *in vivo* performance of the coated alginate/gellan/ locust bean gum beads, a gamma scintigraphic study was carried out. The scintigraphy of the optimized formulation CF3 (92 ± 0.8% Radio labelling efficiency) filled in enteric coated capsule was performed using rabbits (animal model) in order to establish its colon targeting potential. From the Scintigraphy images (Figure 3) it can be interpreted that the beads was completely intact in the stomach up to 2 hrs. Images indicate that when beads of mesalamine was in the small intestine the radioactivity was concentrated in a very small area indicating that little release had occurred. The mean transit time from stomach to colon was found to be 6.0 ± 0.47 hrs. The beads started to disintegrate in colon after 6.0 hrs. Once the beads entered the ascending colon, there was considerable spreading of radioactivity from ascending colon toward the transverse colon which was most likely caused by the action of the bacterial enzymes in the colon degrading the gellan gum and accelerating the release of radioactivity. Scintigram shows the residence of beads in colon more than 12 hrs. These results showed that Eudragit S-100 coated beads formulation may be useful for targeting mesalamine to the colon.

Stability studies

The optimized formulation does not show any significant change with respect to shape, colour, surface and *in vitro* drug release. The results of drug release are shown in the Figure 4.

CONCLUSION

In this study it was found that the gellan gum is a suitable biodegradable and biocompatible natural gum for preparing colon targeted beads of mesalamine and the ionotropic gelation was appropriate technique of prepare the beads of mesalamine. The results of *in vitro* release studies revealed that the coated CF3 Formulation shows the better and prolong release of the mesalamine in the colon. The *in vivo* study on rabbit indicated that the drug release from the formulation take place at its target site. The physical stability studies were not shown any significant change in *in vitro* release of optimize formulation before and after storage period.

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