Original Article

Preformulation Considerations Development and Evaluation of Mesalamine Loaded Polysaccharide-Based Complex Mucoadhesive Beads for Colon Targeting

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ABSTRACT

Background: Inflammatory bowel disease (IBD) is a chronic inflammatory disease of large intestine consisting of ulcerative colitis and Crohn's disease. Mesalamine is a first line drug use for the treatment but it has drawback of low water solubility and low permeability. In addition, oral drug delivery to colon faces the problem of gastric degradation and thus increases dose size and frequency. **Objectives:** The present study was aimed to assay mesalamine and develop an enteric coated mesalamine loaded mucoadhesive beads using guar gum, sodium alginate and carbopol 940 for an effective delivery to colon. **Methods:** Preformulation study was done to check the purity of drug. The beads were prepared by ion gelation methods and coated with Eudragit S100 and characterized. **Results:** The melting point and absorbance maxima of mesalamine were found to be 282°C and 220 nm respectively. The FTIR study confirms the drug and polymers were compatible. A total 5 different formulations were prepared which specifically release the drug in the colon region in sustain release fashion. The formulation MA-F4 was chosen as an optimized formulation. The Particle size, Zeta potential, %EE, %Yield, %DL and *in vitro* drug release of optimized formulation was found to be 445.6 ± 67.1 μm, -28.01 ± 0.16 mV, 94.98 ± 3.22, 96.27 ± 3.22, 30.4 ± 0.9 and 95.07 ± 3.85 respectively. **Conclusion:** The study demonstrated that the prepared beads can release the mesalamine in sustained release manner and helps in management of IBD.

Key words: Inflammatory bowel disease, Colon, Mesalamine, Mucoadhesion, Beads.

INTRODUCTION

Inflammatory Bowel Disease (IBD) is a multidimensional gastrointestinal (GIT) disease. IBD involves major 2 types of inflammatory problems such as Crohn's disease (CD) and ulcerative colitis (UC) which may be either progressive or chronic with unknown etiology.[1-3] The intestinal clinical symptoms associated with UC and CD are abdominal pain, bloody diarrhea, weight loss and vomiting whereas extra-intestinally are mostly affected with eyes, joints, skin and bile duct. IBD have been associated with numerous factors like environment, immune system, micro-organism, GIT microflora and genes. IBD pathogenesis and development is also linked with irregular protein glycosylation.[2,4] If these problems is not early diagnosed and treated, severe intestinal complications can be arisen in the form of fistulae, extreme abdominal pain, colon cancer, bowel function impairment and disability.[5,6] Till date a permanent treatment of IBD is not established therefore the treatment of disease is symptomatic and sometime lifelong. Globally, the occurrence and morbidity of IBD is dramatically growing and influence all ages of population including pediatric and geriatric patients and affect their overall life.[7,8] Currently, epidemiological survey revealed that incidence of IBD

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in developing countries like India is rising which is challengeable task.[9]

An anti-inflammatory drug like 5-amino salicylates (5-ASA) is the first line therapy used for UC and CD. It is safe and well tolerated in most of the cases. 5-ASA drugs, like Sulfasalazine is the first choice drug for the treatment of IBD, mesalamine is another choice of drug used in the treatment of mild to moderate conditions of IBD.[10,11] Corticosteroids, like prednisolone and budesonide are used when aminosalicilates does not give desired results. These drugs are used for management of moderate to severe IBD.[12] Immunomodulators agents, like 6-mercaptopurine, azathioprine and methotrexate, antibiotics like metronidazole, rifaximin, ciprofloxacin and most important anti-TNF-α-antibodies (infliximab, adalimumab, golimumab and certolizumab) also play a significant role in the management of severe disease stages.[3,13-15] Mesalamine is a first line drug for the treatment of mild to moderate IBDs including UC and CD.[16] Patients with IBD exhibit an increased generation of leukotrienes and hydroxy eicosatetraenoic acids through cyclooxygenase (COX) and lipoxygenase (LOX) pathways. It is assumed that mesalamine block the cyclooxygenase and inhibit the production of prostaglandin and thus diminishing the inflammation in the colon.[17] It is pH-dependent drug and can release in the environment of colon, where maximum concentration of drug can be achieved at targeted site.[11,18]

Mesalamine belongs to class IV of BCS which means it has low water solubility and low permeability thus low bioavailability in the cells. This drawback of mesalamine is a major challenge for the formulator to design a suitable delivery system. Targeted oral drug delivery system is pioneering for improving drug efficacy and bioavailability at the site of action with mucoadhesive approach.[19] In the last few decades, a continuous quest has been raised towards colonic delivery of drugs to enhance their therapeutic efficacy at target sites and minimize related side effects. Although, merely a few approaches like colon targeted drug delivery system that has the ability to work in a complex environment of the gastrointestinal tract (GIT) of the human. The polymeric microparticles prepared from polysaccharides are attractive carrier system for colon targeting because of their properties like pH swellability in specific pH and degradability in colonic lumen by bacteria.[20,21] Thus, mucoadhesive drug delivery system is highly required for site specific treatment for IBD which would be fulfilled by micro-particulate carrier system.

Guar gum (GG) is natural gum which has been well explored as a polymer for colon targeting due to its extraordinary gelling efficiency, mucoadhesiveness, biodegradability and sustained release property.[22] Sodium alginate (SA) is a anionic polysaccharide, alginic acid with sodium salt, obtained from seaweeds. The specific feature of SA includes mucoadhesiveness, pH-sensitivity, cross-linking capability, low toxicity and biodegradability that offer more suitability for colon drug targeting.[23] Carbopol 940 (CP) is a high molar mass cross-linked polyacrylic acid polymer having extremely wetting and colloidal viscosity property (1000 times more viscous) which forms a sparkling clear gel in water.[24]

Present study was aimed to assay physiochemical properties of mesalamine and to develop mesalamine loaded mucoadhesive microcarrier for an effective delivery to colon against IBD. The microparticles were coated with a Eudragit S 100 (enteric coating polymer) which dissolve specifically at colonic pH (7.4) and allow the drug to release in the colon region in sustain release fashion. Thus, after oral administration, enteric coated microparticles will be presented in unchanged form in stomach environment but it will be released in alkaline pH of colonic fluid which may be suitable for management of IBD.

MATERIALS AND METHODS

Materials

Mesalamine standard drug was purchased from Yarrow chem, Mumbai, India. Sodium alginate, Guar gum, Eudragit S-100 and carbopol 940 were purchased from CDH, New Delhi, India. Calcium chloride dihydrate were obtained from Qualikems Fine Chem Pvt. Ltd., Vadodara, India. Glacial acetic acid was procured from Merck, Mumbai, India. Analytical grade other reagents and chemical were employed in the experiments. Purified water was employed throughout the experimental procedure.

Preformulation and Physicochemical Characterization of Mesalamine

Before the development of any dosage form, it is necessary to know several physiochemical properties (preformulation studies) of the drug. This information helps the formulator to develop an elegant, stable, effective and safe dosage form. In addition, preformulation study also aids in the authentication of the drug. Several preformulation factors were evaluated for mesalamine before going for formulation of beads.

Melting Point Determination

It is one of the easiest ways to determine the purity of the drug. The melting point of the mesalamine was determined by capillary tube method. The open end

of the melting point capillary was filled with the small amount of the drug and tapped several times so that drug reaches to the bottom of closed end. The capillary was placed in digital melting point apparatus. The apparatus was heated initially at a rate of 10°C/min and then 1°C/min near to the expected melting point. The temperature at which melting of the drug take place was noted down.

UV-wavelength (λ_{max})

Accurately weighed mesalamine drug (10 mg) was dissolved in 10 ml volumetric flask containing methanol. Then volume was adjusted upto the level with sufficient quantity of methanol. This gave the concentration of 1000 µg/ml. It was diluted to 100µg/ml. Further, different aliquots were prepared with the stock solution. These aliquots were analyzed spectrophometrically (Shimadzu, UV-1800, Japan).

Preparation of Standard Curve

Mesalamine (10 mg) was accurately weighed and dissolved in 100 ml volumetric flask containing hydrochloric acid buffer (0.1N HCL, pH 1.2) and simulated gastric fluid (SGF, pH 6.8, pH 7.4) individually. To get a 100 µg/ml concentration in the buffer medium of pH 1.2, pH 6.8 and pH 7.4 volume was maintained upto level separately. This stock solution was used to prepare further standard solution of the drug. From the standard stock solution, different aliquots 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0 ml were taken from different buffer pH and volume made up to 10 ml with each buffer pH 1.2, pH 6.8 and pH 7.4 individually in volumetric flask to produce 2, 4, 6, 8, 10, 12, 14, 16 18 and 20 µg/ml respectively. The solutions were filtered (Whatman filter paper # 41) and absorbance was measured at 220 nm using a double-beam UV-spectrophotometer (Shimadzu, UV-1800, Japan).

Solubility Studies

Solubility is an important preformulation study which helps the formulator to choose the best solvent for the drug and enables the formulation of drug in an appropriate dosage form. The solubility of the drug was determined by taking excess amount of drug in 1ml of a specific solvent in a glass vial and subjected to shaking for 12 h. Further the vial was kept aside till the undissolved drug settle down. The supernatant was collected and drug content was determined spectrophotometrically (λ_{max} 220 nm).

FT-IR Analysis

FTIR spectrum investigation was carried out to confirm the compatibility of pure drug mesalamine with different excipients which was used for preparation of optimized mesalamine loaded mucoadhesive beads. The KBr discs of pure mesalamine, polymers (sodium alginate, carbopol-940, guar gum) and powdered mesalamine brads were prepared and scanned in an FTIR spectrophotometer (Shimadzu IR Affinity, Japan) at 4000-500 cm^{-1} wave number.

Differential Scanning Calorimeter (DSC) Studies

The thermal behavior of mesalamine was studied in Perkin Elmer, Pyris Diamond DSC, USA at a heating rate of 10°C /min. Accurately weight sample (5mg) was sealed in an aluminum pan and measurement was performed at a heating range of 50- 370°C. Before analysis, equipment was standardized with zinc and indium.

Preparation and Coating of Mucoadhesive Beads

Mesalamine loaded mucoadhesive beads were prepared by ionic gelation method as reported by Deshmukh *et al.* (2020) with slight modification. Briefly, sodium alginate (SA 3%, w/v) was dissolved in distilled water (DW). Carbopol 940 (CP; 250, 500, 750, 350 and 150 mg) and guar gum (GG; 500, 500, 250, 150 and 100 mg) were dissolved in 25 ml DW separately and kept in room temperature for 24 h to swell completely. The CP and GG dispersion were mixed together at equal ratio (1:2, 1:1, 3:1, 2.33:1 and 1.5:1 w/w) under magnetic stirrer (Remi, India) at specific stirring rate for 1 h in order to find homogeneous mass of both the gum. Mesalamine (250 mg) was then added in to gum slurry. This gum slurry was added in SA solution and mixed properly at an appropriate stirring rate by using magnetic stirrer as shown in Table 1. Cross linking solution was prepared by dissolving calcium chloride ($CaCl_2$, 5% w/v) in DW containing glacial acetic acid (GAA, 10% v/v).

To ensure the drug and gum mixture was free from air bubbles before its use. Then the drug and gum mixture were added drop-wise to the cross-linking solutionthrough a disposable syringe needle (24G size).

Table 1: Formulation of mesalamine loaded mucoadhesive beads.

Formulation code	Drug: Polymer ratio (mg, w/w)	Polymer ratio (CP: GG) (mg, w/w)	Stirring rate (rpm)
MA-F1	1:1 (250:250)	1.5:1 (150:100)	500
MA-F2	1:2 (250:500)	2.33:1 (350:150)	500
MA-F3	1:3 (250:750)	1:2 (250:500)	500
MA-F4	1:4 (250:1000)	1:1 (500:500)	500
MA-F5	1:4 (250:1000)	3:1 (750:250)	500

The drug loaded micro beads were formed immediately and kept a side for 30 min to complete the reaction. The mesalamine loaded beads were collected by filtration and washed with DW 4-5 times to remove the $CaCl_2$ residues from the beads. Beads were dried under hot air oven at medium temperature 55 for 3 h. Spherical dried mesalamine - mucoadhesive beads were packed into air tight vials and stored in desiccators for further studies. Similarly, the placebo (without drug) mucoadhesive beads were also prepared.

The prepared mucoadhesive beads were coated with an enteric coating solution (Eudragit S 100) for protection of beads to release its content in upper part of GIT as described by Deshmukh *et al.*[25] Briefly, Eudragit S 100, enteric coating polymer (2.98 g) was dissolve in 25 ml mixture of isopropyl alcohol and acetone (4:3). PEG-400 (3% w/v), a plasticizer was also added to the solution. The microparticles were coated by this solution by dip coating method till the weight increase by 10% and further dried in hot air oven at 40°C for 4 h.

Evaluation of Mucoadhesive Beads of Mesalamine

Particle Size and Zeta Potential Analysis

Zetasizer (Nano ZS90, Malvern instruments Ltd., UK) was utilized for size and zeta potential analysis. The mesalamine loaded beads were suspended in distilled water and analyzed in Zetasizer for suitable particle size and zeta potential.

Percentage Drug Entrapment Efficiency (%EE), Drug Loading (%DL) and Yield

UV-vis spectrophotometer (Shimadzu, UV-1800, Japan) was used for this analysis. %EE, %DL and % yield of the mesalamine loaded mucoadhesive beads (MA-F1-5) were determined as describe by Anande *et al.* 2008. About 100 mg of the beads were crushed and made to powder in a glass mortar. Further, to release the drug content the powder was extracted out in SGF (100 ml) by using bath sonicator. Thereafter, the suspension was filtered through membrane filters (0.45 µm) and analyzed spectrophotometrically at 220 nm.[26] Each determination was made in triplicate. Analysis was performed in triplicate (n=3). The %EE, %DL and yield were calculated according to the following equations:

$$\%EE = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} \times 100$$

$$\%\ DL = \frac{\text{Total amount of drug in beads} - \text{Amount of free drug}}{\text{Total weight of beads}} \times 100$$

$$\%\ Yield = \frac{\text{Total weight of beads}}{\text{Total weight of drug, polymer and other non} - \text{volatile solids}} \times 100$$

Swelling Index

The swelling extent of beads (MA-F1-5) was carried out in phosphate buffer of pH 6.8 and 7.4. An exactly weighed 100 mg of beads were transferred to the basket of USP type I dissolution test apparatus and allowed to swell for 12 h at 37 ± 0.1°C. Thereafter, the fluid adhering with beads were wiped out by using filter paper and swelled beads were taken out for weight measurement. Swelling index is calculated according to the following formula.[27]

$$\%\ \text{Degree of swelling}\ (\alpha) = \frac{\omega_s - \omega_0}{\omega_0}$$

Where, ω_o = weight of un swollen beads and
ω_s = weight of swollen beads.

Mucoadhesion Testing by In vitro Wash-Off Method

The mucoadhesive properties of mesalamine-loaded beads (MA-F1-5) were evaluated by the *in vitro* wash-off technique as describe by Malik *et al.*[28] Briefly, goat intestinal mucosa was collected from local slaughter house and a size of 1 × 1 cm was excised and fixed into the glass slide with the help of thread. The beads (100 in count) were then sprinkled over the mucosa and then to an arm of tablet disintegration test apparatus. The samples were subjected to up and down movement in beaker containing 900 ml of phosphate buffer (pH 7.4) maintained at 37 ± 0.5°C. The beads were observed regularly for adherence with intestinal mucosa and total beads adhered were recorded for a period of 12 h. The percentage mucoadhesive efficiency of the beads was recorded according to the formula:

$$\%\ \text{Mucoadhesion} = \frac{\text{Number of adhered of beads}}{\text{Total number of applied of beads}} \times 100$$

Surface Electron Microscopy (SEM) Study

The architecture of MA-F4 beads (shape and surface) was studied by using SEM (Jeol, JSM-1600, Tokyo, Japan). Prior to imaging, the beads were sprinkled over sided adhesive carbon tape which was stuck to aluminum stub. Further it was subjected to gold coating using gold sputter under inert atmosphere in a high-vacuum evaporator. These specimens were analyzed and their photomicrographs were recorded at different magnifications.

In vitro Drug Release Study

The *in vitro* drug release study from novel mesalamine formulations (MA-F1-5) and marketed tablet formulation of mesalamine (Pentasa 250 mg) was performed according to the procedure reported by Anande *et al*. Multiple dissolution rate test (six paddles) apparatus was used for performing the release profile of the formulations. A simulated GI fluid (SGF) was used as the dissolution medium. Mesalamine beads (100 mg) were weighed accurately and put into the beaker containing 900 ml SGF. The dissolution content was rotated at 100 rpm at 37 ± 0.5°C and exact equilibrium (sink) condition was maintained during the study period. The different pH of SGF and GI transit conditions were obtained by changing the dissolution medium at different periods. In the Initial study period, pH 1.2 of SGF was maintained for 2 h with the help of 0.1N HCl. Then accurately weighed quantity of potassium di-hydrogen phosphate (1.7 g) and di-sodium-hydrogen-phosphate-dihydrate (2.2 g) were added in to the SGF medium, adjusting the pH to 6.8 using 1.0 M NaOH and the release rate study was continued for further 2 h. After 4 h, the pH of SGF was changed to 7.4 with 0.1N NaOH for 24 h. A 5 ml sample was taken from the dissolution medium at regular time period and an equivalent volume of fresh SGF was replaced to preserve the sink conditions. Prior measurement, all the test solutions were filtered through the syringe filter (0.45µm, NYL) and analyzed spectrophotometrically at 220 nm.

Stability Study

MA-F4 was tested for stability study according to the guidelines of ICH. It was tested at 25 ± 2 / 60 ± 5 % RH, 30 ± 2 / 65 ± 5 % RH and 40 ± 2 / 75 ± 5 % RH for 180 days. The changes were observed periodically for 45, 90 and 180 days and shelf life were calculated for storage condition at different temperatures.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed for analysis of the experimental data. Graph Pad Prism software-5, San Diego, CA, USA was used for statistical analysis of the data. All the data were determined by the mean ± standard deviation (SD) and mean variations were considered to be significant at $P < 0.05$.

RESULTS

Physicochemical Characterization of Mesalamine

Melting Point

M. P. of mesalamine was found to be 282°C. But in this temperature drug started to decompose.

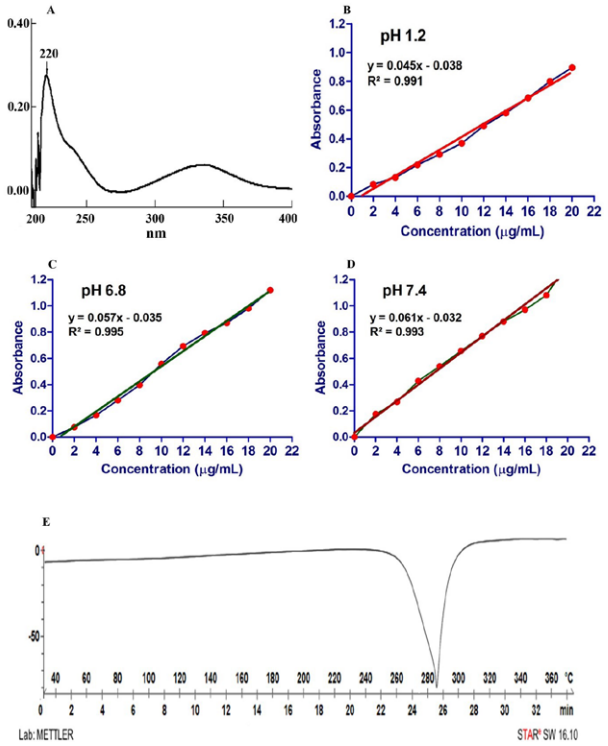

Figure 1: Preformulation of mesalamine. (A) UV-Visible spectra of mesalamine (B) Calibration curve of mesalamine in SGF (pH 1.2) at 220 nm (C) Calibration curve of mesalamine in SGF (pH 6.8) at 220 nm (D) Calibration curve of mesalamine in SGF (pH 7.4) at 220 nm (E) DSC of the mesalamine.

Determination of Absorbance Maxima (λ_{max})

The mesalamine dissolved in methanol and diluted to give solution of 10 µg/ml which was spectrophotometrically analyzed at UV range. Solution gives peak at 220 nm as shown in Figure 1A. Thus, this wavelength was selected as λ_{max} for the further study.

Preparation of Standard Curve

The stock solution of mesalamine was diluted with either hydrochloric acid buffer (0.1N HCl, pH 1.2), SGF pH 6.8, pH 7.4 to get a concentration range of 2 to 20 µg/ml of mesalamine. The absorbance was measured at 220 nm and calibration curve of mesalamine was plotted between absorbance and concentration for the 3 different buffers solutions was shown in Figure 1 B-D. The data were subjected to linear regression and the correlation coefficient was found to be 0.9903 for pH 1.2, 0.995 for pH 6.8 and 0.9943 for pH 7.4. Thus, the value of correlation coefficient indicates that the concentration range of 2 to 20 µg/ml of mesalamine obeys beer's lambert law.

Differential Scanning Calorimeter Studies

The DSC spectrum of mesalamine shows a sharp endothermic peak at 282°C (Figure 1E). This was in accordance to the obtained melting point of the

Table 2: Solubility study of mesalamine in different solvents.

Solvents	Solubility
Distilled water	Insoluble
0.1N HCl solution.	Insoluble
Phosphate buffer of pH 6.8	Insoluble
Phosphate buffer of pH 7.4	Insoluble
Methanol	Soluble
DMSO	Freely soluble
Acetone	Freely soluble

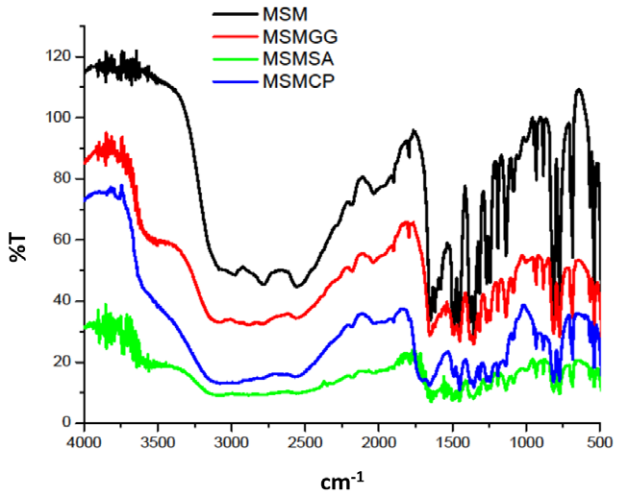

Figure 2: Overlayed FTIR spectrum of mesalamine with the ingredients of formulation. MSM: mesalamine, MSMGG: mesalamine and guargum, MSMSA: mesalamine and sodium alginate, MSMCP: mesalamine and carbopol.

mesalamine. Thus, this study proves the structural stability of the mesalamine.

Solubility Studies

The solubility mesalamine in different solution was given in Table 2.

FTIR Analysis

The drug-excipient interaction FTIR spectrum has shown in Figure 2. Drug sample FTIR spectrum data was interpreted and matched with standard FTIR spectra of mesalamine which confirms the authenticity of the sample drug by identifying peaks as similar as reference drug. The result shows that the mesalamine shows band at 1649.14 cm^{-1} (C=O stretching), 1489.05 cm^{-1} (C=C), 1452.4(CH_2 bending), 1355.96 cm^{-1} (C-N). In addition, FTIR spectrum analysis of drug and components of formulation was also performed. There were very slightly shift of drug in to polymer matrix. Thus, the spectrum proves that the drug mesalamine was quite compatible with its polymer used in development of mucoadhesive beads.

The results of preformulation studies of mesalamine were in accordance to standard and therefore it can be concluded that the drug was authentic. Moreover, the drug was compatible with the other ingredients used for the formulation of the microparticles.

Development of Mesalamine Mucoadhesive Beads

Mesalamine loaded different mucoadhesive beads were developed by an appropriate ionic-gelation technique as describe by Deshmukh *et al.* with slight modifications.[25] Beads were prepared by using different drug: polymer ratios (w/w). Time of cross linking between 5% $CaCl_2$ and sodium alginate-based CP/GG slurry is a key point for formation of spherical micro beads at specific stirring rates under magnetic stirrer. The optimized stirring rate was found to be 500 rpm for fabricating mesalamine loaded appropriate mucoadhesive beads. The beads were then coated with Eudragit S100 until weight of the beads increases by 10%- 12% by its initial weight.

Evaluation of Mucoadhesive Beads of Mesalamine

Particle Size and Zeta Potential Analysis

The dissolution and absorption of a drug from formulation is influence by its particle size. Zeta potential value indicates the stability of the formulation. Therefore, the particle size and zeta potential of the mesalamine loaded mucoadhesive beads of all the formulation (MA-F1 to MA-F5) were analyzed. The size of the beads was found to be in size range of 445.6 ± 67.1µm to 842.1 ± 76.9 µm and zeta potential was found to be in range of -24.14 ± 0.21mV to -31.6 ± 0.26mV. As the concentration of polymer increases the mean particle size also increases Table 3. It is well known that as the particle size decrease the surface area increases which may facilitate the drug absorption from the formulations. Hence, it is desirable that the optimized formulation should have a less particle size for drug to release from the formulation. A zeta potential result indicates that the formed beads are negatively charged and as a result they will have a good stability. The size distribution and zeta potential of MA-F4 was given in Figure 3.

Surface structure and morphology of the optimized formulation, MA-F4 has been visualized through SEM (Figure 4). Photomicrograph of the uncoated particle was rough in shape while the beads coated with Eudragit S-100 were seen as a smooth and spherical in shape. Thus, the coating helps in smoothing the surface of the beads.

Table 3: Characterization of mesalamine loaded different mucoadhesive beads.					
Formulation code	**Particle size (μm)**	**Zeta potential (mV)**	**%EE**	**%Yield**	**%DL**
MA-F1	842.1 ± 76.9	-25.93 ± 0.30	72.28 ± 6.54	74.51 ± 6.45	23.4 ± 0.6
MA-F2	755.4 ± 60.2	-24.14 ± 0.21	91.33 ± 3.63	83.34 ± 5.85	28.5 ± 0.9
MA-F3	457.3 ± 56.7	- 24.92 ± 0.28	82.52 ± 2.14	82.58 ± 4.26	20.8 ± 0.7
MA-F4	445.6 ± 67.1	-28.01 ± 0.16	94.98 ± 3.22	96.27 ± 3.22	30.4 ± 0.9
MA-F5	667.9 ± 86.4	-31.6 ± 0.26	88.43 ± 3.11	87.83 ± 3.18	30.1 ± 0.8

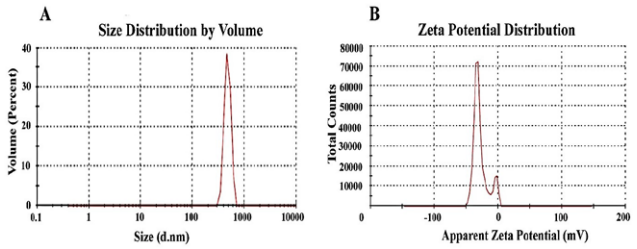

Figure 3: Particle size distribution (A) and zeta potential (B) of the optimized formulation MA-F4.

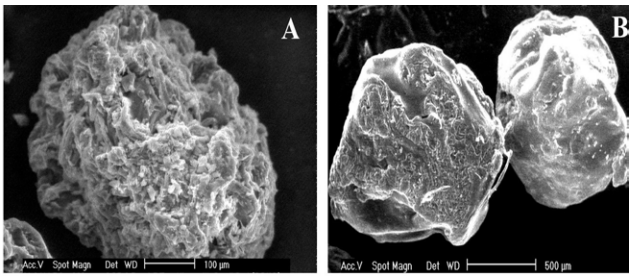

Figure 4: SEM photomicrographs of mesalamine loaded uncoated mucoadhesive beads (A) and mesalamine loaded Eudragit S-100 coated mucoadhesive beads (B).

Drug entrapment Efficiency, Drug Loading and Percent Yield

% EE, % drug loading capacity and % yield of the different formulations, MA-F1-5 were calculated and results have been represented in Table 3. MA-F4 showed highest value than that of others. The entrapment efficiency was found to be 94 ± 3%, drug loading was 94 ± 3% and % yield was 96 ± 3. The formulation MA-F4 contain 1:1 w/w polymer ratio (CP: GG). This ratio was optimal for development of beads at constant 500 rpm. Therefore, the formulation MA-F4 was chosen as an optimized formulation.

Degree of Swelling or Swelling Index (SI)

Swelling efficiency of MA-F1-F5 were evaluated in simulated intestinal fluid at pH 6.8 and pH 7.4 and results were shown in Figure 5(A). Swelling indexes (θ) were found to be in the range of 0.87 ± 0.09 to 1.25 ± 0.05 at pH 6.8 and in range of 0.86 ± 0.05 to 1.28 ± 0.04 at pH 7.4. The best SI (θ) (1.25 ± 0.05 at pH 6.8 and 1.28 ± 0.04 at pH 7.4) value was achieved with MA-F4

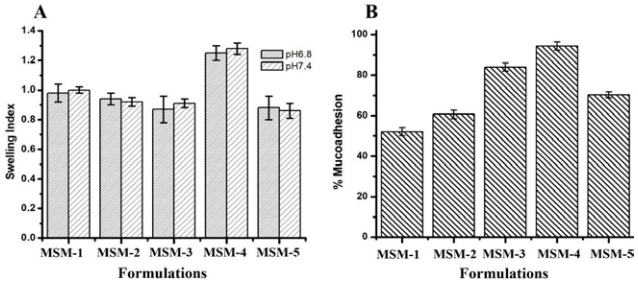

Figure 5: Swelling index (A) and mucoadhesion of mesalamine loaded mucoadhesive beads (B). Values were represented as mean ± SD (*n*=3).

for an extended period (24 h) as compared to other formulations, which is suitable for retaining in colon.

Mucoadhesion

Mucoadhesion of various formulations, MA-F1-5 were performed in goat intestinal mucosa to check out the adhesion efficiency of beads to the intestinal mucosa for prolonged release of drug. Results were shown in Figure 5(B). Mucoadhesion were found to be from 60.67% ± 2.1% to 94.33% ± 2.1% for different formulations. The maximum mucoadhesion (94.33% ± 2.1%) ($P < 0.001$) was achieved with MA-F4 which was statically significantly as compared to other formulations.

In vitro Drug Release

Mesalamine release profile of MA-F1-5 and marketed tablet Pentasa was studied in pH progression medium (SGF) at 37 ± 0.5°C. The maximum mesalamine content was found to be 95.07 ± 3.86 % significantly from MA-F4 than that of other formulations including Pentasa as shown in Figure 6A. Cumulative % release profile from MA-F1-5 exhibited the desired rate as there was no quantifiable drug released at pH 1.2 for 2 h while at pH 6.8 the drug release was quite insignificant for period of 4 h. As represented in Figure 6B, 17.18 ± 2.83% mesalamine was released from Pentasa tablet for 2 h at pH 1.2 but in case of MA-F4 very less amount (>1.60 ± 0.53%) of mesalamine was released for 2 h at pH 1.2. At pH 6.8, 26.01 ± 8.99% drug content was released from Pentasa tablet, while mesalamine was released 3.83 ± 0.43 % from MA-F4 after 4 h. The drug released from beads at acidic pH 1.2 which may be due to

the permeation process through membrane but released in pH 7.4 could follow both matrix erosion and diffusion pattern of drug release. Mesalamine 97.16 ± 3.92% was released from the Pentasa tablet for period of 12 h at pH 7.4 because of uncoated tablet. Formulation MA-F4 exhibited remarkable sustained release profile (95.07 ± 3.85%) for 24 h at pH 7.4 as compared to Pentasa tablet, which may be due to mucoadhesive property of the sodium alginate based carbopol 940 and guar gum coated beads.

Drug Release Kinetics

Different kinetic treatments were applied to interpret the release of mesalamine from different matrices which has been represented in Table 4. The best formulation, MA-F4 followed Korsmeyer's Peppas model having r^2=0.9236 and diffusion exponent value n= 1.6442. So the drug release mechanism was super Case-II transport and *non-Fickian* pattern.[29]

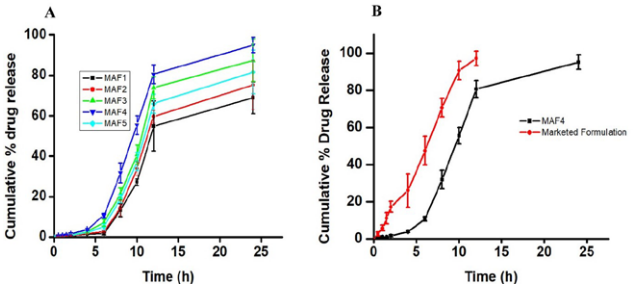

Figure 6: *In vitro* drug release: (A) Release profile of mesalamine loaded mucoadhesive beads (MA-F1-5) at pH 1.2, 6.8 and 7.4. (B) Comparative release profile of optimized beads, MA-F4 and Pentasa tablet at pH 1.2, 6.8 and 7.4. The values were mean ± SD (n = 3).

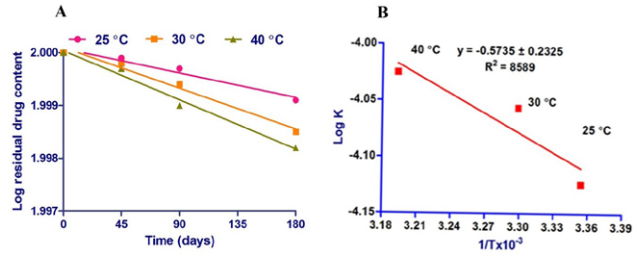

Figure 7: First order (A) and Arrhenius plot (B) of MA-F4 for degradation at different storage temperatures (25°C, 30°C and 40°C).

Stability Studies

The MA-F4 formulation was kept inside amber color bottled and sealed with aluminum foil and evaluated for stability. At a regular interval of 0, 45, 90 and 180 days the formulation was analyzed for its size, zeta potential and residual drug content. Result suggests that the prepared formulation was stable at room temperature. With the increase of temperature there was increase in rate of degradation [first order, Figure 7(A)], decrease in the size and zeta potential. The shelf-life of MA-F4 was predicted from Arrhenius plot and was found to be 3.84, 3.29 and 3.06 years at 25°C, 30°C and 40°C respectively [Figure 7(B)]. The data of particle size and residual drug content at different temperature were also subjected to t-test with 95% significant level and $P < 0.05$.

DISCUSSION

IBD is concerned with GIT problems, particularly small and large intestine consisting of (UC) and (CD). It may be either progressive or chronic with unknown etiology effecting millions of people worldwide and affecting the quality of life of people. Anti-inflammatory drugs especially 5ASA are the first choice of treatment for management of IBD. The oral is the most appropriate and choice of drug delivery with high patience compliances. But the conventional drug delivery has high dose size and dose frequency resulting in increased side effect. Therefore, it is desired that the delivery system should work locally at the colon site or can be systemically absorbed from the colon and simultaneously protecting the drug from exposure to gastric enzymes, altered pH conditions and variations in gastric voiding. Some of the most common approaches used to deliver drug to the colon includes use of polymers releasing drug dependent on pH, time-release coatings, prodrugs and biodegradable polymers.[30-32] In last few years the utilization of polymeric microparticles and beads for colon targeting has become a promising approach.[33] A

Table 4: Kinetic treatment of prepared mesalamine beads formulations.

Formulations	Zero order		First order		Higuchi square root		Korsmeyer plot	
	r^2	k	r^2	k	r^2	k	r^2	n
MA-F1	0.8747	3.2295	0.8846	0.0226	0.7515	15.021	0.8384	1.583
MA-F2	0.879	3.5516	0.8962	0.0269	0.7629	16.603	0.7261	1.6232
MA-F3	0.8778	4.1899	0.8944	0.0396	0.7823	19.859	0.7011	1.4403
MA-F4	**0.8891**	**4.6543**	**0.9249**	**0.0562**	**0.8185**	**22.499**	**0.9236**	**1.6642**
MA-F5	0.8863	3.8803	0.9033	0.0325	0.7818	18.287	0.72	2.3586

wide variety of polymers (natural and synthetic) have gained prominence in pharmaceutical research for developing technology to administer drug directly to the colon. Hence, the present study was aimed to formulate a microparticles carrying mesalamine in the blend of polymer matrix for an effective delivery to colon against IBD. The choice of treatment for IBD involves the use of colon specific anti-inflammatory drug like mesalamine. Yet the actual mechanism of action of mesalamine is doubtful but it was believed that it diminishes inflammation by blocking cyclooxygenase and inhibiting prostaglandin production in the colon.

The anti-inflammatory drug mesalamine was subjected to preformulation studies for its authentication and it was observed that the procured drug has a Melting point and λ_{max} was found to be 282°C and 220nm respectively. DSC study (Figure 1E) gives a sharp endothermic peak at 282°C was similar to MP of drug. In addition, the FTIR spectrum of drug was compared with standard a spectrum which was almost similar. Thus, the various preformulation studies confirm the purity and stability of the mesalamine.

The mucoadhesive beads were prepared by ion gelation technique using 5% acidified $CaCl_2$ solution. Total 5 different batches of formulation (MA-F1 to MA-F5) were prepared and subjected for various characterization and evaluation.

The particle size and zeta potential of the beads was found to be in size range of 445.6 ± 67.1 µm to 842.1 ± 76.9 µm and -24.14 ± 0.21 mV to -31.6 ± 0.26 mV respectively. Beads size is varied with polymeric concentration which has shown in Table 3. The increase polymeric concentration results in increased viscosity of the dispersion which may results in particle size. But it is always desired to have beads with less particle size as they have high surface area. Among all the batches MA-F4 had a least particle size of 445.6 ± 67.1 µm

It is well known that as the particle size decrease the surface area increases which may facilitate the drug absorption from the formulations. Hence, it is desirable that the optimized formulation should have a less particle size for drug to release from the formulation. A zeta potential result indicates that the formed beads are negatively charged and as a result they will have a good stability. Moreover, the charge on the beads confers a good mucoadhesion with the mucosa of the colon via electrostatic interactions which may prolonged the colonic or intestinal residence time for the formulation. The size distribution and zeta potential of MA-F4 was given in Figure 3. The *in vitro* release of mesalamine from different formulations (MA-F1-5) follows the order of MA-F4 > MA-F3 > MA-F5 > MA-F2 > MA-F1. Thus, the formulation MA-F4 exhibit highest drug releases of 95.07 ± 3.86 % in 24 h as compare to other formulations. The smaller particle size (increased surface area) and higher swelling index contributes to the maximum release of drug to the dissolution media. In contrast, formulation MA-F1 having highest particle size of 842.1 ± 76.9 µm exhibits lowest drug release profile as compare to other because of its low surface area, high crosslinking and low swelling efficiency. Therefore, based on results of different parameters the formulation MA-F4 was considered as best one among others. The stability study revealed that the formulation was most stable at 25 ± 2°C and the shelf life (T_{90}) was found to be 3.84 years. At elevated temperature there may be loss of moisture which may be responsible from change in texture, particle size and increase degradation rate. Thus, the mesalamine loaded polymeric shell microparticles coated with Eudragit S 100 can be a promising delivery system for colon targeting. Further, *in vivo* study is required to ensure the colon targeting for management of IBD.

CONCLUSION

IBD is an inflammatory disorder occurred in GIT especially at colon region. There were many conventional drug therapies available for the management and remission of the IBD. But the current conventional drugs delivery system has drawback of high dose size and frequency. Mucoadhesive microparticles played an important role in colon specific drug targeting. It can target the desired site with prolonged release profile for extended periods due to their adhesiveness property to the colonic mucosa. Hence, the objective of the current study was to develop and evaluate mesalamine loaded microparticles (beads) for colon targeting. The beads were developed by a blend of polymer (SA, GG and CP) matrix system of the microparticles. The various preformulation studies help for the identification and establishing the purity of the drug. The beads had good mucoadhesive properties and release its content specifically in the colonic pH in sustained release manner. Hence, mucoadhesive beads would be pioneering in the treatment of IBD. Moreover, it can be good alternative to traditional delivery system which has drawback of high dose and more side effects.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

ABBREVIATIONS

ASA: Aminosalicilates; **BCS:** Biopharmaceutical Classification System; **CD:** Crohn's Disease; **CP:** Carbopol 940; **DL:** Drug Loading; **DSC:** Differential Scanning Calorimeter; **DW:** Distilled Water; **EE:** Entrapment Efficiency; **GAA:** Glacial Acetic Acid; **GG:** Guar Gum; **IBD:** Inflammatory Bowel Disease; **PEG:** Polyethylene Glycol; **SA:** Sodium Alginate; **SEM:** Scanning Electron Microscopy; **SGF:** Simulated Gastric Fluid; **UC:** Ulcerative Colitis.

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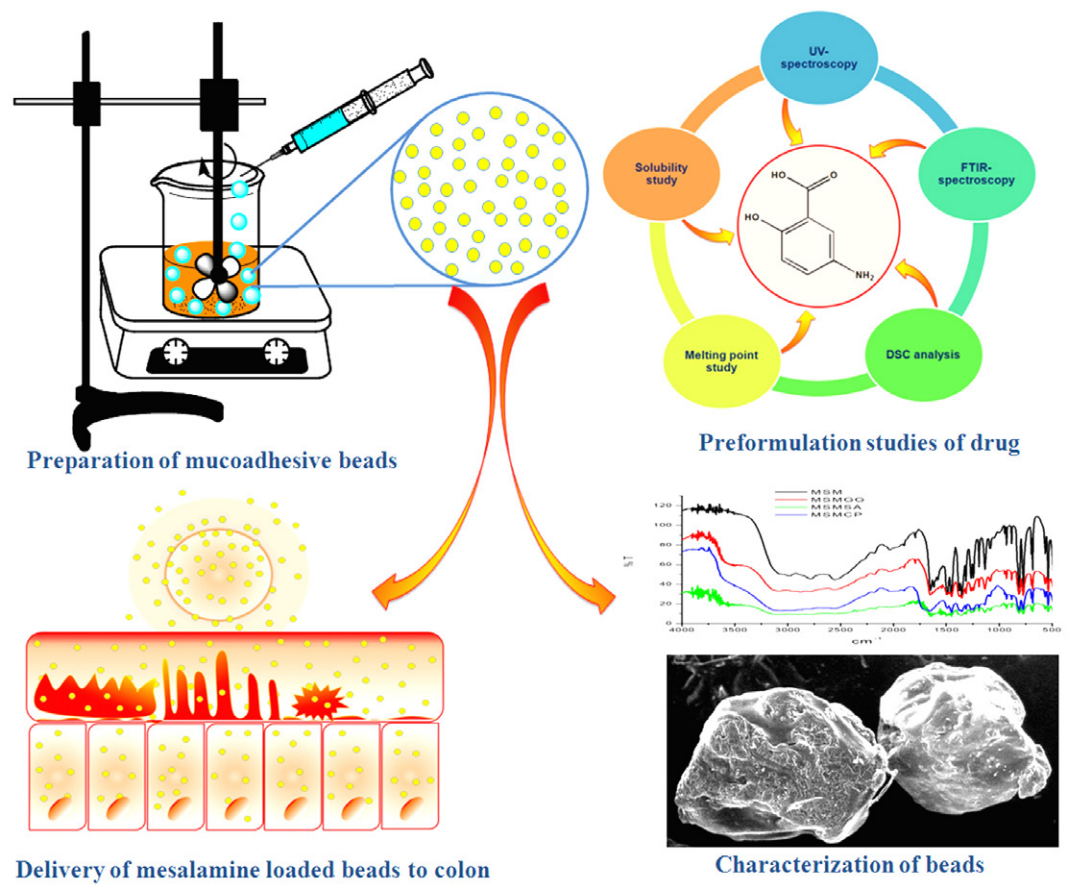
PICTORIAL ABSTRACT



SUMMARY

- Inflammatory bowel disease is a disease of large intestine with clinical symptoms like abdominal pain, bloody diarrhea, weight loss and when untreated leads to fistulae, extreme abdominal pain, colon cancer, bowel function impairment and disability
- Mesalamine is a first line drug use for the treatment but it has low water solubility and low permeability thus low bioavailability in the cells. This problem can be overcome by targeted oral drug delivery system with mucoadhesive approach.
- Present study was aimed to assay physiochemical properties of mesalamine and to develop mesalamine loaded mucoadhesive beads using sodium alginate and guar gum and carbopol as a matrix polymer.
- The beads were prepared by ion gelation technique using 5% acidified $CaCl_2$ solution. Total 5 different batches of formulation (MA-F1 to MA-F5) were prepared and coated with a Eudragit S 100. The beads were subjected for various characterization and evaluation.
- The melting point and λ_{max} of mesalamine was found to be 282°C and 220 nm respectively. DSC study gives a sharp endothermic peak at 282°C was similar to MP of drug and FTIR study confirms the drug and polymers were compatible.
- The Particle size, Zeta potential, %EE, %Yield, %DL of optimized formulation (MA-F4) have was found to be 445.6 ± 67.1 μm, -28.01 ± 0.16 mV, 94.98 ± 3.22, 96.27 ± 3.22 and 30.4 ± 0.9 respectively. The *in vitro* drug release was found to be 95.07 ± 3.85 at simulated gastric fluid (pH 7.4) in a sustained manner for longer periods (24 h).
- The study demonstrated that the prepared beads release the mesalamine in sustained release manner and helps in management of IBD. It can be good alternative to traditional delivery system which has drawback of high dose and more side effects.

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Dr. Rohitas Deshmukh did his master (M.Pharm) in 2008 from Guru Ghasidas Central University (GGV), Bilaspur, Chhattisgarh, India, where he got expertise in development and evaluation of novel and control drug delivery system. He worked on development of novel delivery systems for management of inflammatory bowel disease. He completed his Ph.D. from Malaria Research Group, Indian Institute of Technology-Guwahati, in the field of Immunology and malaria pathology. Presently Dr. Deshmukh is working as an Associate professor at Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India. His major area of research is development of nanocarriers for the treatment of colon cancer. Presently, he has 21 research publications and 2 international book chapters. In the year 2020, he was awarded with best researcher in novel drug delivery system by RULA. He is also in editorial board of various journals of international repute.

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