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Research Article

Formulation and Evaluation of Mesalamine Retention Enema

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Abstract

Ulcerative colitis comes under inflammatory bowel disease (IBD), a disease of colon, with inflammation confined mostly to mucosa characteristically affecting the rectum. Mesalamine has been used in the maintenance of remission in ulcerative colitis and management of mild to moderate active disease. Thus, it is used as the model drug in the present study and to formulate mesalamine retention enema and its comparative evaluation with the marketed product – MESACOL (Mesalamine Rectal Suspension USP). The suspensions were formulated by controlled flocculation method to maintain the sedimentation volume as that of marketed product. Xanthan gum and guar gum were used as suspending agents and Carbopol 934 as flocculation retardant. The formulations were characterized for their appearance, redispersibility, pourability, assay, pH, rheological properties, sedimentation volume, particle size determination, in vitro drug release and short-term stability of the selected formulations. We observe that appearance, redispersibility, pourability and pH were within the acceptable limits. Particle size of the suspensions of almost all the formulations were lying in the range of 60-65μm as that of marketed product. Sedimentation volume and thixotropic behaviour of the suspensions with xanthan gum was good when compared to guar gum. The percentage of drug content was found to be between 94-100% which was within acceptable limits. *In vitro* drug release at the end of 30 min of F2 (100.04%) and F5 (101.91%) were better than marketed product (87.96%). At the end of short-term stability studies (2 months), Formulation F2 indicated no significant change in sedimentation volume, assay, pH, average particle size and redispersibility and hence it is the optimized formulation.

Keywords: Inflammatory bowel disease (IBD), mesalamine, ulcerative colitis, retention enema.

Introduction

Over the past several years there has been a dramatic increase in bowel diseases. Approximately 75% of patients with mucosal ulcerative colitis undergo operative treatment [1].

Irritable bowel syndrome (IBS) is an idiopathic chronic, relapsing disorder containing abdominal discomfort (pain, bloating, distention or cramps) in combination with alterations in bowel habits (diarrhea, constipation or both). With periodical abdominal pain or discomfort, patients note a difference in the frequency or consistency of their bowel movements. Pharmacologic therapies for IBS suggest that relieving abdominal pain and discomfort and improving bowel function [2].

Ulcerative colitis is the colonic mucosal anti-inflammatory disease which is restricted to large intestine and is usually treated with glucocorticoids or salicylates. However, during periods of remission mesalamine is the drug of choice [3,4]. Aminosalicylates were the first line treatment options for mild to moderate active UC. Drugs that contain 5-ASA have been used successfully for decades in the treatment of IBD. 5-ASA differs from salicylic acid only by the addition of an amino group at 5 (meta) position. Aminosalicylates are supposed to work topically (not systemically) in areas of diseased gastro-intestinal mucosa. Up to 80% of unformulated, aqueous 5 - Aminosalicylic acid is absorbed from small intestine and does not reach the distal small bowel or colon in substantial quantities. To conquer the rapid absorption of 5-ASA from the proximal small intestine; a number of formulations have been designed to deliver 5-ASA to various distal segments of small intestine or colon [5].

Mesalazine (Mesalamine)

These are the official names given to 5-ASA. Realizing that 5-ASA is the active moiety in ulcerative colitis, but is not effective orally because of inability to reach the large bowel (it is absorbed in the small intestine). It has been formulated as delayed release preparations by coating with acrylic polymer. The pattern of release over the length of jejunum, ileum and colon differs among the different formulations, but most of them do effectively deliver 5-ASA to the distal small bowel and colon. A daily dose of 2 to 4 g has been found to improve over 50% patients of ulcerative colitis (up to 80% mild to moderate cases). Less than half of 5-ASA released from this preparation is absorbed, acetylated in the liver and excreted in urine. Like sulfasalazine, the primary use of mesalazine is in preventing relapses, though it may also be used to treat mild to moderate exacerbations. Side effects noted are diarrhea; nausea, abdominal pain and head ache, but are mild and less frequent. Rashes and hypersensitivity reactions are rare. It has nephrotoxic potential, because 30-40 % of 5-ASA is released in ileum and absorbed. It is contraindicated in renal and hepatic impairment.

All oral products are designed to release 5-ASA for action in the intestine hence, minimal systemic absorption should occur. Absorbed 5-ASA and its metabolites are excreted in the urine. The majority of 5-ASA leftover in the colonic lumen and is excreted in faeces. The elimination half-life of 5-ASA can range from 2 to 15 h due to the different formulations/routes of the drug.

Rectal forms of 5-ASA

When only the recto-sigmoid or colon area is involved in IBD, direct administration of the active 5-ASA itself in the form of enemas, suppositories or foam might be preferred. The selection among the various preparations should be guided by the proximal extent of the disease. Scintigraphic monitoring demonstrated that enemas distributed the labelled 5-ASA within 0.5 to 2 h from the rectum and sigma up to the transverse colon and partly even to the ascending colon [6]. During steady state urinary recovery of 5-ASA (0-11% of dose) and of acetyl-5-ASA (7-35% of dose) indicated an absorption of around 25%. The volume of the enemas affected the spread: with 100-ml enemas, a better and more consistent distribution was seen than with 30 or 60 ml enemas. 5-ASA from foam will reach the proximal sigmoid colon. A prolonged retention, a more uniform coating of the mucosa and a better acceptance by the patient might be achieved as

compared to enemas [7]. When given as suppositories 5-ASA will be consistently delivered only to the recto-sigmoid region. However, on an average 13% of the maintenance dose of 1.5 g of 5- ASA/day was recovered in the urine of patients with UC [8]. If combined 5-ASA treatment with enemas (4 g/100 ml at bedtime) and Asacol R tablets 800 mg t.d.s. was applied in patients with UC, concentration of 5-ASA in the rectum and descending colon were about 50- and 3-fold higher, respectively, than with oral therapy . Thus, in left sided UC topical formulations of 5-ASA (e.g. enemas, foam) represent the first choice [9]. The objective of the work is to formulate Mesalamine retention enema by controlled flocculation method using Xanthan gum, Guar gum as suspending agents and Carbopol 934 as flocculation retardant and its comparative evaluation with the marketed product – MESACOL (Mesalamine Rectal Suspension USP). Mesalamine is a water insoluble drug and hence it is formulated as a stable aqueous suspension and evaluated.

Materials and Methods

Materials

Mesalamine was obtained from Ipca Labs, Chennai ; Carbopol 934, sodium acetate, potassium acetate, guar gum, sodium meatabisulfite and sodium benzoate were obtained from Loba Chemie, Mumbai. Xanthan gum was procured from Girijan Corporation, Visakhapatnam; potassium metabisulfite from Merck and disodium EDTA from Universal Laboratories, Mumbai.

Methods

Preformulation Studies

Solubility studies of mesalamine The equilibrium solubility of mesalamine was determined in 1N HCl, Distilled water and pH 7.2 phosphate buffer by placing on a gyratory shaker at 150 rpm (Remi Equipments and Instruments, Mumbai). Concentrations were obtained from the respective standard calibration plots.

Drug-excipient compatibility studies The pure drug and its mixture with xanthan gum and guar gum were subjected to FTIR studies separately, after keeping the mixtures at 45 °C for 48 h. In the present study, potassium bromide disc (pellet) method was employed.

Particle size determination of pure drug 50 mg of the drug was added to 2 ml of distilled water. The resultant suspension was shaken to disperse the particles and a drop of it was taken on a glass slide. Cover slip was placed on it carefully without entrapment of any air bubbles. The slide was examined and 100 particles were counted. They were classified according to the size ranges. The procedure was repeated for three times and average was calculated.

Formulation of Suspension

A mucilage was prepared by triturating gums and Carbopol 934 in a small portion of water. Accurately weighed amount of drug was incorporated into the mucilage by trituration. In another portion of water, preservative, complexing agent and soluble salts were dissolved and added to the mucilage. It was triturated to form uniform suspension and the final volume was made up. Different formulations were prepared as shown in Tables 1 and 2.

Table 1: Formulations of mesalamine with xanthan gum

Ingredients (%w/v)	F1	F2	F3	F4	F5	F6	F7	F8
Mesalamine	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67
Carbopol 934	0.125	0.125	0.125	0.125	0.125	0.125	-	-
Sodium acetate	0.82	0.82	0.82	-	-	-	0.82	-
Potassium acetate	-	-	-	0.82	0.82	0.82	-	0.82
Xanthan gum	0.10	0.15	0.25	0.10	0.15	0.25	0.15	0.15
Sodium meta bisulfite	0.5	0.5	0.5	-	-	-	0.5	-
Potassium metabisulfite	-	-	-	0.5	0.5	0.5	-	0.5
Disodium EDTA	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
Sodium benzoate	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
Demineralized water	100	100	100	100	100	100	100	100

Table 2: Formulations of mesalamine with guar gum

Ingredients (%w/v)	F9	F10	F11	F12	F13	F14	F15	F16
Mesalamine	6.67	6.67	6.67	6.67	6.67	6.67	6.67	6.67
Carbopol 934	0.125	0.125	0.125	0.125	0.175	0.175	0.125	0.125
Sodium acetate	0.82	-	0.82	-	0.82	-	0.82	-
Potassium acetate	-	0.82	-	0.82	-	0.82	-	0.82
Guar gum	0.15	0.15	0.20	0.20	0.20	0.20	0.30	0.30
Sodium meta bisulfite	0.5	-	0.5	-	0.5	-	0.5	-
Potassium metabisulfite	-	0.5	-	0.5	-	0.5	-	0.5
Disodium EDTA	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
Sodium benzoate	0.167	0.167	0.167	0.167	0.167	0.167	0.167	0.167
Demineralized water	100	100	100	100	100	100	100	100

Evaluation [10]

Appearance of suspension Various batches of formulations designed were inspected visually for their appearance.Redispersibility

Redispersibility of the suspension was checked by moving the stoppered cylinder upside down until there was no sediment at the bottom of the cylinder.

Pourability The test is carried out on the batches of suspension after mixing to ensure that the final preparation is pourable and will not cause any problem during filling and during handling by the patient.

Assay A measured volume of the suspension (5 ml) was taken into 100 ml volumetric flask, made up to the mark with pH 7.2 phosphate buffer and diluted as required and its assay was performed by measuring the absorbance at 330 nm using UV spectrophotometer (LabIndia, Mumbai).

pH pH of fresh suspensions was measured by digital pH meter.

Rheological properties Rheologic method can help in determining the settling behaviour of suspension. Brookfield viscometers with variable shear stress control can be used for evaluating viscosity of the suspension. Brookfield digital rotational viscometers (LVDV III Ultra Rheometer with cone and plate attachment) were used to measure the rheological behavior of the designed suspensions.

Sedimentation volume (F) and Particle size Distribution Sedimentation volume (F) is a ratio of the final or ultimate volume of sediment (Vu) to the original volume of sediment (Vo) before settling. It can be calculated by following equation.

$F = Vu/Vo$

The suspension was shaken to disperse the particles and a drop of it was taken on a glass slide. Cover slip was placed on it carefully without entrapment of any air bubbles. The slide was examined and 100 particles were counted using a calibrated eye-piece micrometer. They were classified according to the size ranges. The procedure was repeated for three times and average particle size was calculated.

***In vitro* drug release study** 300 ml of freshly prepared test media, pH 7.2 phosphate buffer was placed in dissolution flasks of USP type II dissolution test apparatus. Six samples each of 1.5 ml of the formulation equivalent to 100 mg of the drug was added to the six jars and the temperature and rpm were maintained at 37 ± 1°C and 50 rpm respectively. Aliquots of samples (5 ml) were withdrawn at 5, 10, 15 and 30 minutes and they were filtered. Absorbances of all the samples were noted at 330 nm in UV spectrophotometer using dissolution medium as blank. Percent mesalamine dissolved was calculated.

Stability In any rational drug design or evaluation of dosage forms for drugs, the stability of the active component must be a major criterion in determining their acceptance or rejection.

Stability of a drug can be defined as the time from the date of manufacture and the packaging of formulation, until its chemical or biological activity is not less than a predetermined level of labeled potency (90%) and its physical characteristics have not changed appreciably. ICH specifies length of study and storage conditions [11].

Long term testing: 25 ± 2° C and 60 ± 5% RH for 12 months

Accelerated testing: 40 ± 2° C and 75 ± 5% RH for 6 months

Stability studies were carried out at 30 ± 2°C, 60 ± 5% RH and 40 ± 2° C, 75 ± 5% RH for 2 months.

Results and Discussion

Pre-Formulation Studies

Solubility studies Solubility studies were performed and absorbance values were tabulated as shown in Table 3.

Table 3: Standard absorbance of mesalamine in 1N HCl, distilled water and pH 7.2 phosphate buffer solutions

	1N HCL		Distilled Water		pH 7.2 Phosphate buffer	
S.No.	Conc. (μg/ml)	Absorbance (λmax-298nm)	Conc. (μg/ml)	Absorbance (λmax-330nm)	Conc. (μg/ml)	Absorbance (λmax-330nm)
1.	2	0.0458	2	0.050	4	0.080
2.	10	0.1060	4	0.095	6	0.122
3.	20	0.2118	6	0.143	8	0.157
4.	30	0.3154	8	0.179	12	0.233
5.	40	0.4268	12	0.264	16	0.315
6.	50	0.5946	16	0.341	20	0.436

Compatibility studies Compatibility studies were performed by using IR spectrophotometer. The IR spectra of pure drug and physical mixture of pure drug and polymers were studied by making a KBr disc. The peaks obtained in spectra of each formulation correlates with peaks obtained in pure drug spectrum. The spectral details are shown below in Figures 1-3.

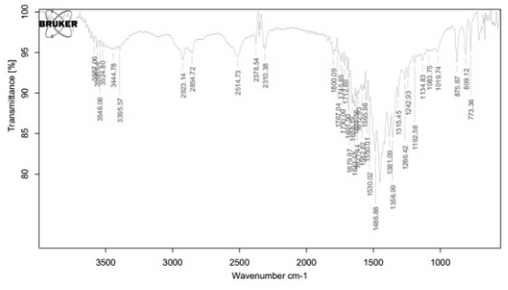

Figure 1: IR spectrum of pure drug (mesalamine)

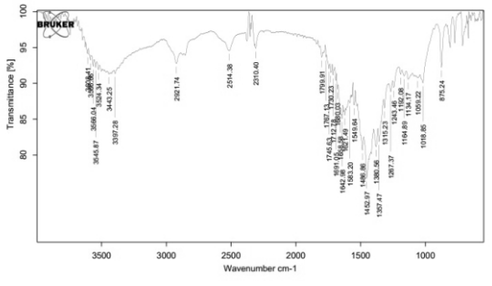

Figure 2: IR spectrum of physical mixture of guar gum and mesalamine

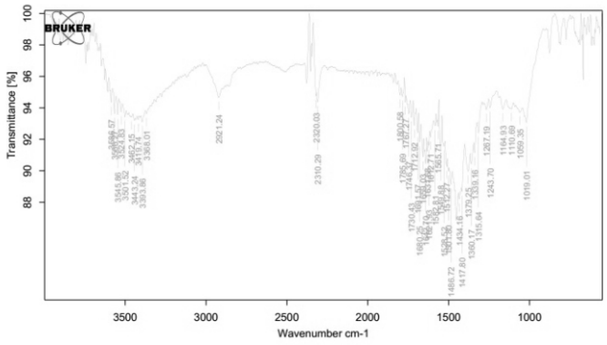

Figure 3: IR Spectrum of physical mixture of xanthan gum and mesalamine

Evaluation of Mesalamine Suspension

Appearance, redispersibility, pourability were found to be good for all the designed formulations.

The percentage of drug content was found to be between 94- 100% of mesalamine which was within acceptable limits. pH of all the formulations was found to be in the range of 4-5 which was within acceptable limits. The results are shown in Table 4.

Sedimentation volume The Mesalamine suspensions were kept in a graduated cylinder after preparation. The heights of sediments were measured at 5, 15, 30 and 60 day intervals. The sedimentation volume F was calculated and tabulated as shown in the Table 4.

Particle size determination Particle size of the suspensions were measured by optical microscopy and mean particle size of almost all the formulations were lying in the range of 60-65μm as that of marketed product. No significant increase in particle size was observed during the short-term stability studies.

Table 4: Evaluation parameters of mesalamine suspensions

Formulations	Avg. Particle size(μm)	Redispersibility	Sedimentation volume (ml)	pH	Assay (%)
F1	64.17±0.11	2 shakes	0.72	4.88	97±1.41
F2	62.15±0.60	3 shakes	0.94	4.24	100±0.67
F3	63.21±0.37	4 shakes	1.00	4.68	96±0.41
F4	62.61±0.37	2 shakes	0.98	4.39	98±3.10
F5	61.23±0.28	3 shakes	0.98	4.21	99±0.83
F6	63.82±1.73	4 shakes	0.64	4.10	97±2.31
F7	62.21±1.37	2 shakes	0.55	3.73	96±1.23
F8	64.51±0.40	2 shakes	0.65	3.73	96±0.56
F9	65.60±0.95	3 shakes	0.72	4.26	95±1.31
F10	61.02±2.18	3 shakes	0.72	4.62	94±0.95
F11	62.92±0.38	3 shakes	0.72	4.72	96±3.14
F12	65.61±0.73	3 shakes	0.75	4.91	96±3.65
F13	61.27±0.36	4 shakes	0.76	4.53	95±4.97
F14	63.19±1.30	4 shakes	0.75	4.57	96±0.81
F15	66.67±0.26	3 shakes	0.68	4.81	97±0.63
F16	64.54±1.83	3 shakes	0.68	4.71	96±3.03
MP	60.12±1.52	3 shakes	0.94	4.63	98±0.43

Sedimentation volume The Mesalamine suspensions were kept in a graduated cylinder after preparation. The heights of sediments were measured at 5, 15, 30 and 60 day intervals. The sedimentation volume F was calculated and tabulated as shown in the Table 4.

Particle size determination Particle size of the suspensions were measured by optical microscopy and mean particle size of almost all the formulations were lying in the range of 60-65μm as that of marketed product. No significant increase in particle size was observed during the short-term stability studies.

Rheological Properties Viscosities of all the formulations were recorded with the help of Brookfield viscometer by varying shear rate starting from 10 sec^{-1} to 200 sec^{-1} and then back again to 10 sec^{-1}. The rpm was increased at intervals of 5 from 5 to 100 and then decreased at the intervals of 5 at room temperature. Among the formulations using xanthan gum as suspending agent (F1 to F8), F2 and F5 showed viscosities comparable to the marketed product. The results were plotted and the rheograms are shown in Figures 4 to 6.

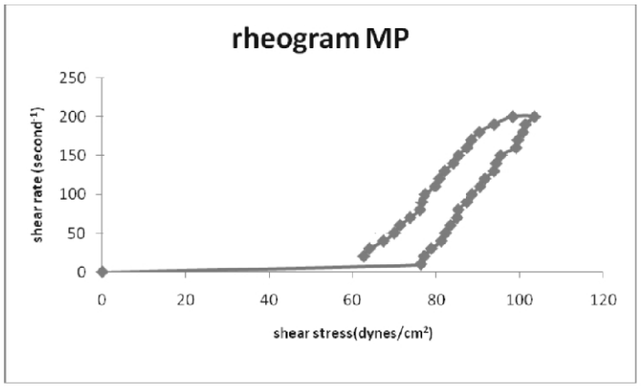

Figure 4: Rheogram of formulation MP

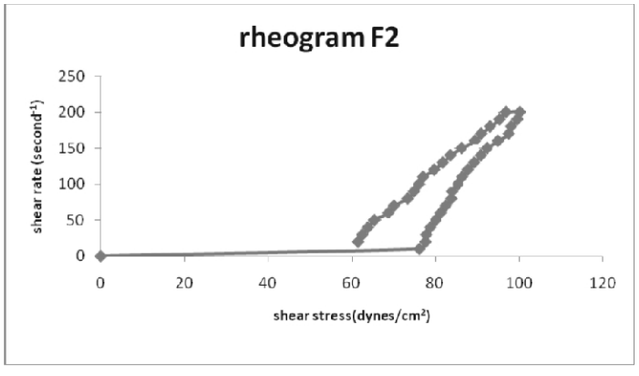

Figure 5: Rheogram of formulation F2

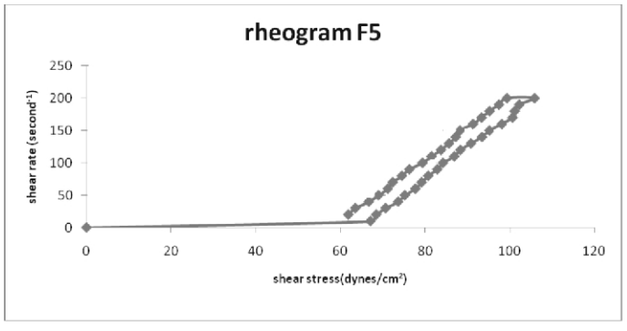

Figure 6: Rheogram of formulation F5

***In vitro* drug release** The In vitro drug release study for all batches was carried out in USP type II dissolution test apparatus up to 30 minutes using pH 7.2 phosphate buffer. The plot of percent cumulative drug release versus time (min) was depicted in Figure 7.

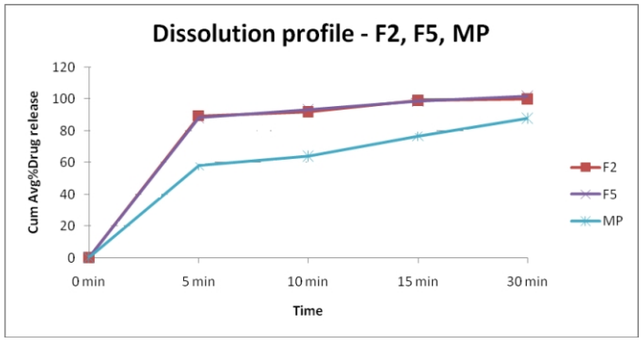

Figure 7: Dissolution profiles of formulations F2, F5, MP

From the results, it was observed that, the formulations with xanthan gum F1 to F8 had shown better drug release than formulations with guar gum F9 to F16. The formulations F7 and F8 had shown poor drug release properties. This may be due to lack of Carbopol 934 in those formulations which acts as flocculation retardant so that the drug release from the settled sediment was slower than the remaining formulations which contained Carbopol 934. Among the formulations with guar gum, F9 to F16, an increasing concentration of guar gum from 0.15%w/v to 0.20% w/v decreased the drug release. So, concentration of Carbopol 934 was increased from 0.125% w/v to 0.175% w/v in order to improve percent drug release. Although percent drug release was improved, there was no improvement in sedimentation volume. So another two formulations (F15 and F16) were tried with 0.30% w/v guar gum. The % drug release was improved but still not better than F2 and F5. Out of all formulations, F2 and F5 showed better release (100.04% and 101.91% respectively) than the marketed product (87.96%).

Stability studies of selected formulations The selected formulations were placed in 60 ml amber coloured bottle. The packed bottles were placed in stability chamber maintained at 30 ± 2° C/60± 5% RH and 40±2°C/75±5% RH for 2 months. Samples were collected at 0, 15, 30 and 60 days. The analysis comprised of testing quantifiable parameters possibly change during storage, such as particle size, pH, drug content, sedimentation volume and redispersibility.

Conclusion

From the above results we can conclude that Mesalamine retention enema formulations prepared with xanthan gum as viscosity enhancing and suspending agent showed acceptable properties like sedimentation volume, viscosity, pH, particle size , redispersibility and in vitro drug release characteristics which remained unchanged upon storage for 2 months. However, the formulation F2 using xanthan gum as suspending agent and Carbopol 934 as flocculation retardant proved to be the formula of choice, since it showed the highest drug release and lower lag time when compared to the marketed formulation (MESACOL). So, mesalamine rectal suspension can be used for rectal drug delivery in treatment of ulcerative colitis so as to reduce the side effects of drug in stomach and also to local targeting of the drug.

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Declaration of Interest

The authors declare no conflict of interest.

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