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Formulation And Evaluation Of Colon Targeted Drug Delivery Of Mesalazine (5-Aminosalicylic Acid/ Mesalamine) By Use Of Ph Depended Approarch

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ABSTRACT

The present study focuses on the Formulation and evaluation of a mesalazine mucoadhesive and ph-dependent colon-targeted drug delivery system for the effective management of inflammatory bowel diseases. The objective was to ensure minimal drug release in the stomach and small intestine while providing a controlled, targeted release in the colon. Various batch formulations were prepared using different ratios of mucoadhesive polymers such as pectin etc and ph-dependent polymers such as carbopol 940 to achieve this Colon Targeted Drug Delivery system. The selection criteria emphasized achieving minimal drug release in the first 4-5 hours to bypass the stomach and small intestine and provide protection from upper GIT, followed by a significant release after 5-6 hours for precise colon targeting. Dissolution studies were conducted on nine batches to determine the release profile. Among the tested formulations, Batch 6,7,8 and 9 demonstrated minimal release during the initial 5 hours and a gradual, sustained release thereafter. Batch B3, B6 and B9 showing a higher mucoadhesion profile. Batch 9 the optimal candidate for ph-dependent colon-targeted delivery and as well as muchoadhesion, ensuring effective site-specific drug release. However, Batch 9 stood out with a superior balance of controlled release with high muchoadhesion, confirming its suitability for colon targeting. In contrast, Batches 1, 2, 3, 4, and 5 showed premature drug release within the first 3-4 hours and batch B6, B7 and B8 it's a showing less mucoadhesive profile compare to batch 9 failing to meet the criteria for ph-dependent delivery and mucoadhesive properies. The study concludes that Batch 9 is the best formulation for a mesalazine mucoadhesive and ph-dependent colon-targeted drug delivery system, offering enhanced therapeutic efficacy and reduced systemic side effects in the treatment of colonic disorders. This optimized system leverages controlled release technology to address the limitations of conventional therapies.Further investigations, including in vivo studies, are recommended to validate the in vitro findings and confirm the clinical potential of this formulation. The promising results from this study the way for advanced, targeted drug delivery systems for chronic gastrointestinal diseases.

Key Words:

Mesalazine, pH-dependent drug delivery, Colon-targeted tablets, Carbopol 940, Pectin, Compression coating, Inflammatory bowel disease, Delayed release, Mucoadhesion test.

Introduction

In the past few decades, the prevalence of colonic disease has increased worldwide, calling for effective treatment strategies for colonic disorders to ensure safe and effective medical therapy. Colon-targeted drug delivery is essential for the treatment of site- specific diseases such as Crohn's disease (CD), colorectal cancer, ulcerative colitis and amoebiasis. Ent amoeba Histolytic, a unicellular protozoan parasite, causes infections in the large intestine and leads to the disease amoebiasis. The prevalence of infection by E histolytic is so high that there are approximately 34-50 million cases of symptomatic infection annually. According to the WHO report on epidemic diseases, infection by E. Histolytic results in 100000 deaths worldwide each year, making amoebiasis the second leading cause of death due to protozoan parasites after malaria. Any drug delivery system's have principal objective is to deliver a therapeutic dose of medication to the target site such that the required drug concentration can be quickly attained and subsequently maintained. Drugs might be administered locally or systemically at the colon. Mesalazine (5-aminosalicylic acid) is widely used as a first-line treatment for colonic disease such as ulcerative colitis due to its anti-inflammatory properties, helping to maintain remission and prevent disease progression. The efficacy of mesalazine largely depends on its ability to reach the colon in sufficient therapeutic concentrations while minimizing systemic absorption and side effects. Mesalazine (5-aminosalicylic acid, 5-ASA) exerts its therapeutic effects primarily through anti-inflammatory, immunomodulatory and antioxidant mechanisms, acting locally in the colonic mucosa. It inhibits the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reducing the production of pro-inflammatory prostaglandins and leukotrienes. Additionally, mesalazine suppresses nuclear factor-kappa B (NF-κB), leading to decreased levels of key pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), which play a crucial role in the pathogenesis of ulcerative colitis. Furthermore, mesalazine has antioxidant properties, scavenging reactive oxygen species (ROS) and reducing oxidative stress-induced mucosal damage. It also modulates the immune response by decreasing the activation of T-cells and macrophages, thereby preventing excessive immune-mediated tissue injury. These combined mechanisms contribute to the reduction of inflammation, tissue damage and relapse in ulcerative colitis, making mesalazine a cornerstone in the long- term management of the disease.[1,2,4]

Conventional oral formulations often result in premature drug release, leading to suboptimal therapeutic effects and potential gastrointestinal side effects. To overcome these limitations, colon-targeted drug delivery systems have gained attention, as they ensure site-specific drug release, thereby enhancing therapeutic efficacy while reducing adverse mesalazine have low system absorption and high local absorption. Among the various approaches, Mucoadhesive and pH-dependent drug delivery systems offer a promising strategy by combining controlled release mechanisms with prolonged retention in the colonic mucosa, leading to improved drug absorption and prolonged therapeutic action. In this study, we aim to develop and evaluate a novel mesalazine Mucoadhesive and pH-dependent colon-targeted drug delivery system designed to enhance drug localization to the ileocolic region. [3,5,6] The formulation involves the use of specialized polymers that enable a dual mechanism: pH-dependent release for initial drug protection and Mucoadhesive properties for prolonged colonic residence time. Various formulation batches with different polymer ratios are optimized to achieve the desired drug release profile while ensuring complete drug release within the targeted site. In vitro dissolution studies, swelling index analysis, and Mucoadhesion strength testing are employed to assess the formulation's performance. Additionally, in vitro release kinetics are analysed to determine the drug release mechanism and its suitability for colonic targeting. The development of such an advanced drug delivery system is crucial in addressing the increasing burden of colonic diseases, offering a potential improvement in treatment outcomes for patients suffering from ulcerative colitis. By enhancing mesalazine's site-specific delivery, this approach can help achieve higher drug concentrations in the inflamed colonic mucosa, leading to better disease management and improved patient adherence. Ultimately, the findings of this study can contribute to the advancement of targeted drug delivery technologies, providing a foundation for future research in the field of gastroretentive and colon-targeted therapeutic systems.[7,8,9]

MATERIALS AND METHODS MATERIALS

MATERIALS

Mesalazine (5-aminosalicylic acid, 5-ASA) is the active pharmaceutical ingredient (API) used for its anti-inflammatory properties, specifically in the treatment of ulcerative colitis and colonic diseases. The formulation includes various excipients that ensure proper drug delivery by improving stability, bioavailability, and patient compliance. Ethyl cellulose is used as a pH-dependent release polymer for colon targeting, while pectin and sodium alginate serve as Mucoadhesive polymers for prolonged retention and controlled release. Starch acts as a binder and diluent, lactose increases bulk and improves compressibility, and microcrystalline cellulose (MCC) enhances tablet hardness and disintegration. Sodium starch glycolate acts as a superdisintegrant, Polyvinylpyrrolidone (PVP K30) improves tablet cohesion as a binder, magnesium stearate functions as a lubricant to reduce friction during compression, talc acts as a glidant to enhance powder flow, and polyethylene glycol (PEG) serves as a plasticizer and film-coating agent. Solvents and reagents are vital in formulation preparation and analytical testing. Dissolution media simulate different pH conditions in the stomach, intestines, and colon. Hydrochloric acid (0.1N HCl) is used as a dissolution medium for simulated gastric fluid (SGF), phosphate buffer (pH 6.8) simulates intestinal conditions in simulated intestinal fluid (SIF), and simulated colonic fluid (SCF) is used for colon-targeted drug release studies. Sodium hydroxide (NaOH) is used for pH adjustments in dissolution media, while potassium dihydrogen phosphate aids in buffer solution preparation. Methanol serves as a solvent for UV-visible spectrophotometry, ethanol is used in standard calibration curve preparation, and distilled water acts as a blank in spectrophotometric analysis.

All materials were stored under appropriate conditions to maintain their stability and quality. The API was stored in a cool, dry place at 2-8°C. Polymers were stored at room temperature in tightly sealed containers to prevent moisture uptake. Reagents and solvents were stored in designated cabinets according to their material safety data sheets (MSDS).

METHODS [10]

DEVLOPMENT OF CALIBRATION CURVE BY USING U.V-SPECTROPHOTOMETER [11,12]

Calibration curve of Mesalazine using 0.1 N HCl :

Preparation of standard stock solution: Weigh and transfer about 100mg of Mesalazine and 100ml 0.1NHcl in a 100ml volumetric flask.

Preparation of test solution from stock solution :

Take 10ml and dilute to 100 ml with 0.1NHcl stock solution ƛ max is calculated by using concentration in the Range of 200- 400 nm.

Standard graph Of Mesalazine :

Weigh and transfer about 100 mg of Mesalazine into 100 ml volumetric flask, Add 0.1NHcl solution, make up the volume to 100 ml with 0.1NHcl solution. From the stock solution, take 10ml and dilute to 100ml with 0.1NHcl, working solution is prepared. From the stock solution 2,4,6,8 and 10µg/ml were prepared and the absorbance was measured at 232 nm.

Calibration curve of Mesalazine using pH 6.0 Phosphate buffer :

10mg of drug was dissolved in phosphate buffer pH 6.0 and final volume was making up to 100ml volumetric Flask. The stock solution concentration was 100µg/ml obtained. It was diluted with phosphate buffer pH 6.0 to Obtain solution in concentration range 2,4,6,8,10µg/ml. Absorbance of µg/ml solution was measured between 200-400nm by using spectrophotometer.

Calibration curve of Mesalazine using pH 7.2 Phosphate buffer :

Accurately weighed 10 mg of drug, dissolved in sufficient volume of phosphate buffer pH 7.2 and then made Volume up to 100 ml with phosphate buffer pH 7.2 and then working solutions of different concentrations 2,4,6,8,10(μ g/ml) were prepared. The absorbance was obtained at λ max 301nm and calibration curve was Plotted between concentration and absorbance.

PRE-FORMULATION STUDIES

Powder flow properties

Preformulation studies are the initial phase of drug development where the physical, chemical, and mechanical properties of the drug substance (API) and its compatibility with excipients are evaluated. These Preformulation studies provide critical data for the design, development, and optimization of a stable and effective dosage form. Pre- Formulation Studies. It involves the comprehensive evaluation of the physicochemical properties of a drug substance (Active Pharmaceutical Ingredient, API) and its compatibility with excipients. These studies are essential for designing a stable, effective, and manufacturable dosage form.

Bulk Density

Bulk density is the ratio of the mass of a powder to its bulk volume, which includes the volume occupied by the powder particles and the void spaces between them. This parameter helps assess how the powder fills a given volume under normal conditions.

Formula: Bulk Density Mass of powder (g) /Bulk Volume (mL)

Weigh a specific amount of powder. Pour the powder into a graduated cylinder without tapping, Record the volume. Bulk density is crucial to determine the storage and handling properties of powders. It indicates how much powder can be stored in a given volume, which affects packaging and manufacturing costs. It also helps in predicting the powder's flow ability and compaction during tabletting.

Tapped Density

Tapped density refers to the ratio of the mass of a powder to its volume after tapping or compacting, which reduces the void spaces between particles. This gives an idea of the powder's packing behaviour under mechanical stress.

Formula: Tapped Density = Mass of powder (g) /Tapped Volume (mL)

Weigh a specific amount of powder. Pour it into a graduated cylinder and tap it (e.g., 100- 200 taps) until the volume becomes constant, Record the tapped volume. Tapped density is an indicator of the powder's packing potential. It helps in understanding how well a powder will compact in tablet formation.

Carr's Index (Compressibility Index)

Carr's Index is a measure of a powder's tendency to compact or collapse. It is calculated as the difference between bulk and tapped densities, and it is a key indicator of flow ability and compressibility.

Formula: Carr's Index (%) = Tapped Density – Bulk Density /Tapped Density times 100

The Carr's Index helps evaluate how easy it is to handle and process powders during manufacturing. A lower Carr's Index indicates that the powder has better flow ability and is less likely to clump or form bridges during processing. A higher Carr's Index means the powder is more likely to compress and form agglomerates, which can interfere with consistent mixing and tabletting.

Hausner Ratio

The Hausner Ratio is a ratio of the tapped density to the bulk density, providing insight into the flow properties and compressibility of a powder.

Formula: Hausner Ratio = Tapped Density /Bulk Density

The Hausner Ratio is used as an indicator of a powder's flowability. A Hausner Ratio of ≤ 1.25 suggests good followability, while a value above 1.25 indicates poor flowability. This parameter is important for ensuring that powders behave consistently during manufacturing processes like mixing, granulation, and tabletting. helps predict the powder's behaviour under mechanical stress and its ability to form uniform tablets.

Angle of Repose

The angle of repose Is the maximum angle formed between the surface of a pile of powder and the horizontal plane. It is a direct measure of the powder's flowability, indicating how easily a powder can flow when poured.

Formula: Angle of Repose (0)=tan-1 height/radius

H= height of the powder pile.
R-radius of the powder pile's base. Units: Degrees

The angle of repose helps assess the flowability and cohesiveness of powders. A lower angle of repose (25-30°) indicates excellent flow, while a higher angle (40° and above) suggests poor flow. This parameter is important for determining how easy it is to handle and process a powder during tablet manufacturing, especially in processes like granulation and filling of capsule or tablet molds.

PREPARATION OF CORE TABLET [13,14]

The preparation of core tablets begins with the accurate weighing of ingredients, including Mesalazine (API) and excipients such as microcrystalline cellulose (MCC), sodium starch glycolate, PVP K30 (as a binder), magnesium stearate, and talc. The dry ingredients, specifically Mesalazine, MCC, and sodium starch glycolate, are then blended homogeneously to ensure uniform distribution.
Next, a binding solution is prepared by dissolving PVP K30 (5 mg per tablet) in water or ethanol, mixing until a clear solution is obtained. During the wet granulation process, this binding solution is gradually added to the dry mixture while continuously mixing to form a cohesive, damp mass, ensuring it is wet enough to form granules but not overly saturated. The damp mass is then passed through a 16-mesh sieve to produce granules of uniform size. These wet granules are dried in a tray dryer or oven at 50°C for 30 minutes to remove excess moisture. Once dried, the granules are further sieved through an 18- mesh sieve to break up any lumps and maintain consistency. The dried granules are then lubricated by mixing them with magnesium stearate and talc to improve flow properties and prevent sticking during compression. Finally, the lubricated granules are compressed into core tablets using a tablet press with a 0.5 cm die, ensuring that each tablet has a weight of 250 mg.

Table-1 Composition of core tablet

Materials	Quantity (Per Tablet)
Mesalazine (API)	200 mg
MCC (Microcrystalline Cellulose)	30 mg
Sodium Starch Glycolate	10 mg
PVP K30	5 mg
Magnesium Stearate	2 mg
Talc	3 mg

PREPARATION OF COATING TABLET BY COMPRESSION COATING [15]

The preparation of the coating layer begins with accurately weighing the required quantities of all ingredients, including Carbopol 940, starch, pectin, lactose, sodium alginate, and PEG. These ingredients are then passed through a fine mesh, such as a 40- mesh sieve, to achieve uniform particle size. Once sieved, they are thoroughly mixed in a mortar or blender for 10-15 minutes to ensure homogeneity. To begin the coating process, half of the prepared coating blend (approximately 125 mg) is placed into the die of a tablet press. The core tablet (weighing around 250 mg) is then positioned centrally over this initial coating layer in the die. The remaining half of the coating blend (125 mg) is added on top of the core tablet, ensuring even coverage. The entire assembly is then compressed using a tablet press to form a uniformly coated final tablet. Once compressed, the final tablets are inspected for defects and collected for further testing or packaging.

Table -2 Composition of coating Materials

Material	Quantity (Per Tablet)
Carbopol 940 (Ph-dependent polymer)	As per below table (5.3-6)
Starch (filler)	60 mg
Pectin (mucoadhesive polymer)	As per below table (5.3-6)
Lactose (filler)	40 mg
Sodium Alginate (film-forming agent)	5 mg
Polyethylene Glycol (PEG, plasticizer)	5 mg
Total Coating Weight	250 mg

Table-3 Formulation batches with different Carbopol 940 and pectin concentrations

Batch No.	Carbopol 940	Pectin
B1	Low (50 mg)	Low (20 mg)
B2	Low (50 mg)	Medium (40 mg)
B3	Low (50 mg)	High (60 mg)
B4	Medium (100 mg)	Low (20 mg)
B5	Medium (100 mg)	Medium (40 mg)
B6	Medium (100 mg)	High (60 mg)
B7	High (150 mg)	Low (20 mg)
B8	High (150 mg)	Medium (40 mg)
B9	High (150 mg)	High (60 mg)

EVALUATION [16,17]

PHYSICAL EVALUATION

The evaluation of mesalazine Mucoadhesive and pH-dependent colon-targeted drug delivery tablets is a process designed to ensure that the formulation meets its therapeutic objectives and Rationale of localized drug delivery with minimal systemic side effects. By employing a the combination of pH-dependent and Mucoadhesive Approaches, these tablets achieve both prolonged retention in the colon and controlled drug release. The success of this approaches systems depends on achieving a balance between the physical, chemical and biological properties of the formulation. Additionally, the evaluation must confirm that the tablet provides a lag phase to match gastrointestinal transit times, followed by site-specific release in the colon. This ensures the drug remains stable in the stomach's acidic pH and is released efficiently in the alkaline environment of the colon. Such evaluations are critical for optimizing therapeutic outcomes and meeting regulatory requirements for safety, efficacy and quality. It includes physical and chemical evaluation.

Size and Shape

To ensure uniformity in size and shape for consistent dosing, ease of handling and patient acceptability. To verify that tablets comply with pharmacopoeial standards for size and shape.
Procedure: Select a random sample of 10 tablets from the batch. Measure the diameter, thickness and shape of each tablet using a Vernier calliper or micrometer. Record observations for any irregularities or deviations in size or shape.

Calculation

Average the diameter and thickness of the tablets: Average Diameter (or Thickness) Sum of individual measurements/ Number of tablets

Percentage Deviation Measured Size - Standard Size/ Standard size times 100.
Significance: Ensures consistent tablet size for proper packaging and dosing. Uniform size and shape improve patient compliance by making the tablets easier to swallow And more aesthetically pleasing.

Organoleptic Property

To evaluate the appearance, colour, taste and odor of tablets, which impact patient acceptability and product identity. To detect any variations that may indicate formulation or stability issues.

Procedure: Visually inspect a sample of tablets under adequate lighting for colour uniformity, surface, Texture and general appearance. Assess the odor of the tablets by sniffing gently, ensuring the test is conducted in a clean, odor-free environment. For taste evaluation, dissolve a small fragment of the tablet in water (if applicable), ensuring it Is safe to taste.
Significance: Organoleptic properties ensure product consistency and help identify potential issues with stability or formulation Good appearance, pleasant taste and acceptable odor enhance patient compliance, especially for pediatric or geriatric formulations.

Weight Variation

Weight variation is an essential test to ensure the uniformity of the amount of drug and excipients in each tablet. This test verifies that the tablets contain the correct amount of active pharmaceutical ingredient (API) and excipients within acceptable limits, ensuring proper dosing for the patient.

Procedure : A sample of 20 tablets is taken from a batch for testing. Each tablet in the sample is individually weighed using an analytical balance with a high degree of precision (usually accurate to 0.0001g).

Calculation: Calculate the average weight of the 20 tablets by adding the weight of all tablets and dividing By 20. Determine the individual tablet weight and compare it with the average weight. The variation in weight should not exceed the permissible limits, which are usually specified By pharmacopoeias (e.g., USP, IP).

Weight Variation Limits: For tablets weighing more than 324 mg, the weight variation should be within ±5% of the average weight. For tablets weighing 324 mg or less, the variation should be within ±10% of the average weight. Tablets that fall outside these limits are considered non-compliant and are rejected.

Hardness

Hardness is the force or pressure required to break or crush a tablet. It measures the tablet's strength and structural integrity and ensuring that tablets can withstand mechanical stress during handling, packaging and transportation without breaking, yet disintegrate efficiently when ingested.

Procedure: A sample of tablets (usually 10) is selected.
Tablets are tested for hardness using a tablet hardness tester (e.g., Monsanto Hardness Tester, Schleuniger or Pfizer Tester).The hardness tester applies a force to the tablet and measures the pressure required to break it. The force is recorded in kilograms (kg) or Newton (N).

Calculation: The hardness of individual tablets is measured and the average hardness Calculated.

Hardness Range: The required hardness typically falls within a range of 4 to 8 kg (depending on the type of tablet). For chewable tablets, the hardness may be lower, while for coated tablets, the hardness may be higher. Tablets with insufficient hardness may break easily, whereas too hard tablets may not disintegrate properly during digestion affecting the drug release and absorption.

Friability

Friability is a measure of the tablet's resistance to mechanical wear or damage during handling. Packaging, and transportation. This test is especially important for tablets that will be subjected to physical stress before reaching the patient.

Procedure : A sample of 10 tablets is selected from the batch for ttesting Test Apparatus: The tablets are placed in a friabilator, a rotating drum that simulates the mechanical stress during handling. The friabilator rotates at a set speed (usually around 25-30 rpm) for 100 rotations (about 4 minutes).After the rotation, the tablets are carefully weighed again to determine how much weight was lost due to chipping or abrasion.

Calculation: The percentage of weight loss is calculated using the formula: Friability% Initial weight – Final weight Initial weight times 100

Friability Limits: The acceptable friability limit is typically less than 1% for most tablets. This indicates that the tablets are robust enough to resist breakage during packaging and handling. Tablets with more than 1% weight loss during friability testing may be considered too fragile and require formulation adjustments.

CHEMICAL EVALUATION

Drug Content Uniformity Test Using UV-Visible Spectroscopy

The drug content uniformity of mesalazine tablets was evaluated using UV-Visible Spectroscopy. For the preparation of the standard solution, an accurate quantity of mesalazine (10 mg) was weighed using an analytical balance and dissolved in 100 mL of phosphate buffer (pH 7.4) or methanol to obtain a 100 µg/mL stock solution. This solution was further diluted to obtain a working standard solution of 10 µg/mL, and a UV scan was performed in the 200-400 nm range to confirm the maximum absorbance (Amax) at ~330 nm. A calibration curve was then prepared by measuring the absorbance of different concentrations ranging from 2-20 µg/mL.
For the preparation of the test solution, ten tablets were weighed to determine the average tablet weight. The tablets were then crushed, and a sample equivalent to 10 mg of mesalazine was transferred to a 100 mL volumetric flask. Phosphate buffer (pH 7.4) was added, and the mixture was sonicated for 15-20 minutes to ensure co0mplete drug extraction. The solution was then filtered through a 0.45 µm membrane filter to remove excipients, and appropriate dilutions were made to match the calibration curve concentration range (typically 10 µg/mL).For the measurement of absorbance, the UV- Vis spectrophotometer was set to Amax = 330 nm. The instrument was zeroed using blank solvent (phosphate buffer pH 7.4), and the absorbance of the sample solution was recorded. The drug concentration was then determined by comparing the absorbance value with the calibration curve.

The drug content (%) was calculated using the formula:

Drug Content (%) = Observed Drug Concentration / Labelled Drug Concentration\times 100
According to the USP and IP acceptance criteria, the drug content should be within 85%- 115% of the labelled claim. If the drug content falls within this range, the formulation meets the uniformity criteria. However, if the variation exceeds the limits, formulation adjustments may be necessary. This test ensures that each dosage unit delivers the correct mesalazine dose, maintaining consistency for effective colon targeting.

In vitro dissolution studies

The in-vitro dissolution study is conducted to evaluate the drug release profile of the Mesalazine Mucoadhesive and pH-dependent colon-targeted drug delivery system under simulated gastrointestinal conditions. The objective is to ensure that drug release occurs specifically in the colon, minimizing premature release in the stomach and small intestine. The study is performed using the USP Dissolution Apparatus II (Paddle Method), which is suitable for solid oral dosage forms like tablets. A pH shift method is employed to simulate the transit of the dosage form through different regions of the gastrointestinal tract. In this method, the dissolution media is adjusted to different pH conditions to mimic the stomach (pH 1.2, 0–2 h), small intestine (pH 6.8, 2–5 h), and colon (pH 7.4, after 5 h). Samples of 5 mL are withdrawn at predefined time points (1, 2, 3, 4, 5, 6, 8, 10, and 12 hours), diluted, filtered, and analysed using UV-Vis Spectrophotometry at 330 nm. The cumulative percentage drug release is plotted against time to generate the dissolution profile. For interpretation, the drug release criteria include ≤10% release in the gastric (0–2 h) and intestinal (2–5 h) phases, ensuring minimal premature release, and ≥90% drug release in the colonic phase (after 5 h) within 12 hours, confirming targeted drug delivery to the colon.

According to USP/BP guidelines, the formulation must meet the above acceptance criteria to be considered effective for colon-specific delivery. This in-vitro dissolution study plays a crucial role in validating the Mucoadhesive and pH-dependent drug delivery system. If the dissolution profile aligns with the target criteria, it confirms that the formulation effectively delivers Mesalazine to the colon, improving therapeutic efficacy for ulcerative colitis and Crohn's disease while minimizing systemic side effects.

Ex Vivo Mucoadhesion Test

The Ex Vivo Mucoadhesion Test is crucial for evaluating the adhesive strength of Mucoadhesive drug delivery systems. This test measures the force required to detach the formulation from mucosal tissue, indicating the potential for prolonged retention at the colonic site, controlled release, and enhanced therapeutic efficacy in ulcerative colitis treatment. The objective of this test is to determine the Mucoadhesive strength of the mesalazine Mucoadhesive colon-targeted formulation using ex vivo mucosal tissue.

The materials required include mesalazine Mucoadhesive tablets, freshly excised goat or porcine intestinal mucosa, physiological saline solution (0.9% NaCl), simulated colonic fluid (pH 6.8), a Mucoadhesion testing apparatus (e.g., modified balance or texture analyser), petri dishes, filter paper, adhesive tape, distilled water, and weights or force application devices. In the methodology, a small sheet of mucosal tissue is fixed onto a glass slide using adhesive tape and moistened with simulated colonic fluid (pH 6.8) to mimic mucosal hydration. The mesalazine tablet is then placed onto the moistened tissue, and a 50 g weight is applied for 1-2 minutes to ensure proper adhesion For detachment force measurement, the upper probe of a texture analyser (or a weight pan in a balance setup) is used to apply a vertical force until the tablet detaches from the tissue, and the detachment force is recorded in grams (g). The mucoadhesive strength

(N) is calculated using the formula:

Muchoadhesive strength (N) = Force(g)×G.81 /1000

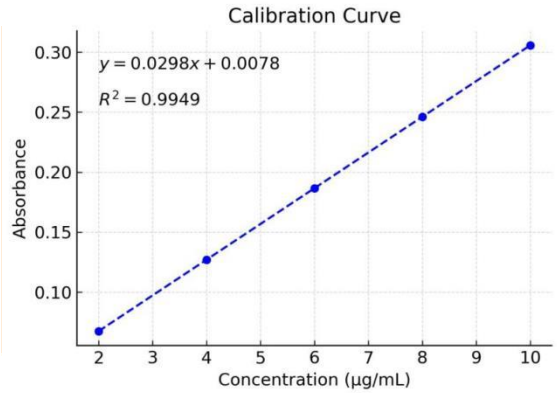
Where Force (g) is the weight required to detach the tablet, 9.81 is the acceleration due to gravity (m/s^2), and 1000 is the conversion factor to Newtons (N). Observations are made by recording the detachment force

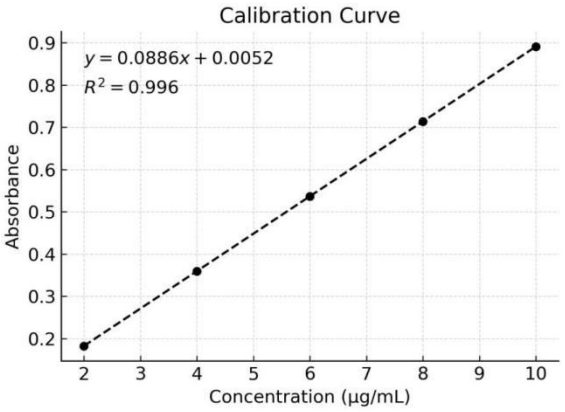
for each sample, and the average Mucoadhesive strength is calculated, where higher values indicate better adhesion. Precautions include ensuring tissue freshness and proper hydration, avoiding excessive hydration that may weaken adhesion, and performing measurements promptly to prevent tissue degradation. The Ex Vivo Mucoadhesion Test effectively evaluates the adhesive strength of the mesalazine Mucoadhesive formulation, providing insights into its potential for colon-targeted drug delivery. Consistently high Mucoadhesive strength confirms prolonged retention and effective drug delivery in the colonic regiol.

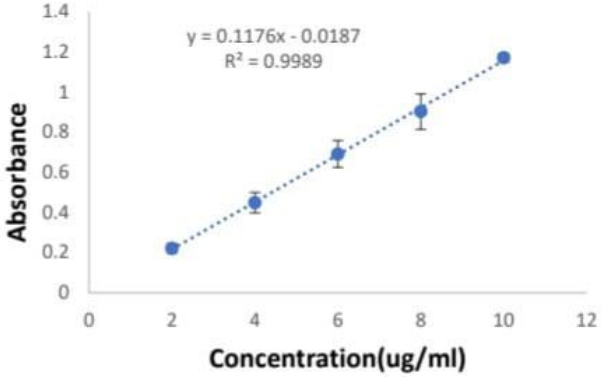
RESULTS

Standard calibration curve of Mesalazine

Linearity studies







Pre-Formulation of Ingredients

Results of pre-formulation evaluation found to be passed according to its standard value.

Table-4 Pre-Formulation of Ingredient

Excipient	Bulk Density (g/mL)	Tapped Density (g/mL)	Angle of Repose (°)	Carr's Index (%)	Hausner's Ratio	Inference (Flow Properties)
Microcrystalline Cellulose (MCC)	0.41	0.56	38.65	36.58	1.36	Poor flow
Sodium Starch Glycolate	0.50	0.65	35.40	23.07	1.30	Moderate flow
PPK 30 (Povidone K30)	0.40	0.43	32.59	7.5	1.08	Excellent flow
Magnesium Stearate	0.25	0.30	26.56	17.49	1.21	Good flow
Talc	0.28	0.42	38.88	32.78	1.48	Very poor flow
Carbopol 940	0.20	0.40	50.00	50.00	2	Very poor flow
Starch	0.30	0.45	40.00	33.33	1.50	Poor flow
Lactose	0.41	0.53	24.44	21.9	1.28	Good flow
Sodium Alginate	0.40	0.60	40.00	33.33	1.50	Poor flow
Polyethylene Glycol (PEG)	0.35	0.50	40.50	30.00	1.42	Poor flow
Pectin	0.30	0.50	40.50	30.00	1.42	Poor flow

Evaluation of tablets

1. Size & Shape

Table-5 Size & Shape

Parameter	Observation
Diameter (cm)	1.0 cm
Thickness (mm)	5 mm
Shape	Round

2. Organoleptic Property

Table-6 Orgenoleptic Property

Parameter	Observation
Color	Uniform
Odor	Normal

3. Results of evaluation of Mesalazine tablets found to be passed from all evaluation tests.

Table -7 Evaluation of tablets

Formulation	Weight Variation (mg)	Hardness (kg/cm³)	Thickness (mm)	Friability (%)
F1	495.6 ± 15.6	6.05 ± 0.13	4.85 ± 0.11	0.52 ± 0.06
F2	510.4 ± 14.9	5.92 ± 0.12	4.80 ± 0.14	0.54 ± 0.05
F3	515.8 ± 16.4	6.10 ± 0.14	4.95 ± 0.16	0.50 ± 0.07
F4	505.2 ± 14.1	5.88 ± 0.11	4.78 ± 0.09	0.48 ± 0.08
F5	517.6 ± 15.7	6.25 ± 0.15	4.90 ± 0.10	0.53 ± 0.06
F6	520.8 ± 17.2	6.00 ± 0.13	5.00 ± 0.14	0.55 ± 0.07
F7	505.4 ± 14.8	6.12 ± 0.12	4.80 ± 0.08	0.51 ± 0.09
F8	520.2 ± 18.4	5.95 ± 0.13	4.98 ± 0.12	0.47 ± 0.10
F9	530.6 ± 20.2	6.30 ± 0.16	5.05 ± 0.19	0.57 ± 0.11

Drug Content Uniformity Test Results

1. Concentration Calculation (X in µg/mL)
Using the Calibration equation:
Y = 0.1176 X - 0.0187
X = (Y + 0.0187) / 0.1176
Where,
- Y = Absorbance

- X = Concentration in µg/mL

2. Drug Content (mg) Calculation

Drug Content (mg) = Concentration (µg/mL) × Dilution Factor

3. Drug Content (%) Calculation

Drug Content (%) = (Drug Content (mg) / 200) × 100

Table-8 Drug Content Uniformity Test

Tablet No.	Absorbance (Y)	Concentration (µg/mL)	Drug Content (mg)	Drug Content (%)
1	1.25	10.79	197.2	98.6
2	1.3	11.08	202.2	101.1
3	1.22	10.61	194.8	97.4
4	1.35	11.48	209.6	104.8
5	1.28	10.91	198.9	99.4
6	1.24	10.71	196.2	98.1
7	1.33	11.3	206.4	103.2
8	1.29	11.0	200.5	100.2
9	1.2	10.44	190.2	95.1
10	1.32	11.32	204.6	102.3

Conclusion

• The drug content percentage ranges from 95.1% to 104.8%, which is within the acceptable limit (85%–115%).

• This confirms that the formulation meets the drug content uniformity test as per USP and IP standards.

• No formulation adjustments are required, and the drug content is suitable for effective colon targeting.

In-vitro Drug Release Study of Mesalazine Formulations

Table-9 Dissolusion test

Time (hours)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Batch 7	Batch 8	Batch 9
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	4	3	2	5	1	3	2	2	2
5	17	16	9	11	19	7	7	5	8
6	25	22	20	17	22	17	15	9	10
7	40	41	38	25	25	29	20	15	15
8	51	48	44	35	32	40	27	18	22
9	71	55	65	57	49	64	35	31	30

10	75	69	73	65	56	69	59	38	45
11	80	73	84	77	65	70	68	61	65
12	82	79	89	85	70	88	80	78	79

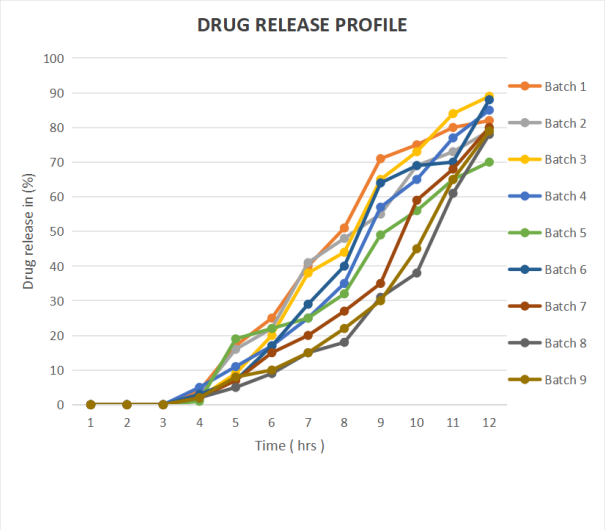


Figure-1 Drug release profile

Ex-vivo Mucoadhesion test

Table-10 Mucoadhesion Test

Batch	Detachment Force (g)	Mucoadhesion Strength (N) (Mean ± SD, n=3)
B1	25.56	0.25
B2	39.41	0.39
B3	57.52	0.57
B4	27.56	0.27
B5	41.21	0.41
B6	53.87	0.53
B7	26.32	0.26
B8	40.26	0.40
B9	62.04	0.62

CONCLUSION

The developed pH-dependent colon-targeted drug delivery system for Mesalazine successfully achieved Targeted site-specific drug release, minimizing release in the stomach and small intestine while maximizing release in the colon. The selection criteria focused on minimal drug release in the first 4-5 hours, followed by a significant release after 5-6 hours to ensure targeted delivery.

Batch Evaluation:

Best Batch for Colon Targeting: Batch 6,7,8 and 9 demonstrated the most effective and controlled release profile, exhibiting a drug release in the first 5 hours and a gradual, sustained release in the colonic environment. It met all criteria for pH-dependent colon targeting.

Not Suitable Batches: Batch 1, Batch 2, Batch 3, Batch 4 and Batch 5 showed premature release, which could lead to absorption in the upper GI tract, making them unsuitable for colon-targeted delivery. Batch 1, 2, 4, 5, 7 and batch 8 have the less mucoadhesive properties.

The Ex Vivo Mucoadhesion Strength Test confirmed the mucoadhesive properties of the formulation, ensuring prolonged retention in the colonic region. Batch 9 effectively protected the drug from early release, enabling controlled and sustained delivery in the colon.

Overall Conclusion:

The formulation strategy combining mucoadhesive and pH-dependent mechanisms successfully delivered Mesalazine to the colon with minimal release in the upper GI tract. Batch 9 was identified as the most optimized formulation, providing an effective treatment option for inflammatory bowel diseases. This project demonstrates the potential of the developed system for colon-specific drug delivery, enhancing therapeutic efficacy and patient compliance. Further in vivo studies are recommended to confirm the in vitro results and evaluate clinical performance.

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