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FORMULATION AND *IN-VIVO* EVALUATION OF MESALAMINE MICROSPONGES FOR COLON TARGETING

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Colon targeting, Quasi emulsion solvent diffusion method, Biphasic release kinetics, Probiotic culture medium, Pharmacokinetics studies, Pharmacodynamic studies, Histopathological study, Macroscopic studies

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ABSTRACT: The present study aims to develop and characterize colon-targeted mesalamine microsponges for treating inflammatory bowel disease, requiring long-term therapy. Mesalamine microsponges were prepared with different ratios of eudragit S100, by quasi-emulsion solvent diffusion method and evaluated. The drug release from *in-vitro* dissolution for all the prepared microsponges was extended to 12 hrs and above, and release kinetics followed a biphasic pattern with Higuchi non-fiction diffusion predominantly. SEM images clearly showed drug entrapment effectively in the microsponges based on the % yield, entrapment efficiency, drug content, and complete drug release (12-15 hrs). Microsponges prepared with a drug-to-polymer ratio of 5:1 (ES5) were considered suitable formulations. Microsponges were equivalent to a dose of 500 mg and were compressed into core tablets by direct compression. All the tableting parameters complied with the official compendia test for tablets as per IP. As the compression of the microsponges did not alter the release, tablets were further subjected to compression coating with cellulose acetate phthalate for colon targeting. From *in vitro* dissolution studies, drug release from the compression-coated microsponge tablets was extended for a period ranging between 30-36 hrs, and in a probiotic medium, complete drug release was obtained between 30-34 hrs due to the presence of microflora. Drug-excipient compatibility studies using FTIR, XRD, and DSC indicated no interactions. Compression-coated tablets of microsponges prepared with eudragit S100 (CT-ES5) showed faster release of drug i.e. in 30 hours inclusive of desired 6 hrs lag time, and were selected for carrying out in vivo evaluation. Notable changes were not observed after long-term and accelerated stability studies according to ICH guidelines for India. ES5 microsponges were equivalent to 5 mg of mesalamine were compressed into mini core tablets of 2 mm diameter and were further compression coated with 10 mg of CAP to obtain compression coated tablet (RCT-ES5) having a diameter of 4 mm for administration to rat. As dissolution profiles of RCT-ES5 and CT-ES5 were superimposable RCT-ES5 was tested in rats for pharmacodynamic activity. Colonic tissue sections from RCT-ES5 treated rats markedly reduced the induced disease, as observed from macroscopic and histopathological studies. Predominant local action can be confirmed for RCT-ES5 when compared to pure mesalamine. The superiority of compression-coated mesalamine microsponge tablets for colon targeting is thus obtained.

INTRODUCTION:

Microsponges [1, 2]: Microsponge drug delivery system has many favorable characteristics, such as enhanced stability due to a high degree of cross-linking; reduced side effects due to targeted and modified drug release; protects the entrapped active ingredients from physical and environmental degradation, which makes it a suitable drug delivery carrier. Microsponges can also deliver active pharmaceutical ingredients efficiently at a minimum dose to the targeted site, reducing severe systemic degradation.

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The size of the microsponges ranges from 5-300 µm in diameter, and a typical 25 µm sphere can have up to 2,50,000 pores. The microsponge technology was developed by Won in 1987, and the original patents were assigned to Advance Polymer

Systems, Inc. The system was employed to improve the performance of topically applied drugs. These consist of porous, non-destroyable structures through which active ingredients are released in a controlled manner. These microsponges with actives can be incorporated into formulations such as tablets, capsules, creams, gels, lotions, and powders and share a broad package of benefits.

Methods of Preparation: Microsponges can be prepared using suspension polymerization in a liquid–liquid system and emulsion systems. The most common emulsion system used is oil-in-water (o/w), in which the emulsion solvent diffusion (ESD) method produces the microsponges.

Liquid-liquid Suspension Polymerization: The porous microsponge can be prepared by the suspension polymerization method in liquid-liquid systems. In this method, the monomers are first dissolved along with active ingredients in a suitable solvent solution of monomer and then dispersed in the aqueous phase, which consists of additives like surfactant, and suspending agents to facilitate the formation of suspension.

Polymerization is then initiated by adding a catalyst or increasing temperature or irradiation. This polymerization process forms a reservoir type of system with a spherical structure. After completion of this step, the solvent is removed by evaporation, producing spherical microsponges.

Quasi-emulsion Solvent Diffusion [3, 4]: Microsponges are also prepared by a quasi-emulsion solvent diffusion method (two-step process) using an internal and external phase. The internal phase contains a polymer dissolved in an organic solvent, and the drug is added slowly to the polymeric solution.

Plasticizer is added to aid plasticity. The internal phase, when poured into the external phase with 8 hours of stirring, due to diffusion of organic solvent out of the droplet and removal of organic solvent from the system, microsponges are obtained. These can be separated by filtration followed by washing and drying in a hot air oven.

Advantages of Microsponge Drug Delivery Systems:

1. Suitable for extended-release having good compatibility with different vehicles and ingredients.
2. Having high entrapment efficiency of up to 80 to 90%.
3. Having a free-flowing character and are suitable for direct compression.
4. Improved thermal, physical, and chemical stability.
5. Suitable for developing novel product forms.
6. Microsponge systems are non-irritating, non-mutagenic, non-allergenic, and non-toxic.

Characteristics of Active Moieties Loaded in the Microsponges:

- It should be water-insoluble or only slightly soluble.
- It should not damage the spherical structure of the microsponges.
- It should be stable in contact with the polymerization catalyst and in polymerization conditions.
- It should be inert to monomers and should not increase the mixture's viscosity during formulation by liquid-liquid suspension polymerization.

Applications of Microsponges [5-7]: Microsponges can be used in various applications. They are used mostly for topical and oral administration and have recently been used in bone and tissue engineering. Due to the high loading capacity of microsponges, they can be used as excipients in drug and cosmetic products.

Topical Delivery of Microsponges: Microsponges are widely used as topical preparations, especially in cosmetics, due to their elegance. Microsponge systems can prevent excessive accumulation of ingredients within the epidermis and enhance the safety, effectiveness, and aesthetic quality of topical prescription, over-the-counter, and personal care products.

Oral Delivery of Microsponges: Microsponges are suitable for oral drug delivery, as these systems increase the release rate of poorly water-soluble drugs by entrapping in the pores of microsponges. These carrier systems bind to the rough surface of the intestinal mucosa and increase the rate of adsorption and dissolution with an enhanced rate of bioavailability. Microsponge carrier systems having a size less than 200 μm that the macrophages can efficiently take up, exhibit effective localized drug action at the desired site. These are easily compressible and produce mechanically strong tablets.

Microsponges used in Bone and Tissue Engineering: Artificial grafts solve several problems by compounding a collagen-microsponge with a biodegradable polymeric scaffold composed of polyglycolic acid knitted mesh, reinforced on the outside with woven polylactic acid. Biodegradable polymer with collagen microsponge serves as a new bioengineered cardiovascular prosthesis and produces autologous tissue regeneration in cardiovascular surgery [46].

Intramuscular injection of fibroblast growth factor incorporated in a collagen sponge sheet gave a sustained release in the mouse sub-cutis according to the biodegradation of sponge matrix and exhibited local angiogenic activity in a dose-dependent manner. A thin biodegradable hybrid mesh of synthetic poly (DL-lactic-co-glycolic acid) and a tissue-engineered patch made of biodegradable polymer and collagen-microsponge provided good in situ regeneration at both the venous and arterial wall, suggesting that this patch could be used as a novel surgical material for the repair of the cardiovascular system.

MATERIALS AND METHOD: Mesalamine was received as a gift sample from Agro Chemicals Ltd., Hyderabad, India, while Eudragit S100, are gift sample from Hetero Labs, Hyderabad, India. Polyvinyl alcohol and ethanol were procured from Loba Chemie, and Qualigens Fine Chemicals, Mumbai, India, respectively. All other ingredients used were of analytical grade and were used as procured.

Fourier Transform Infrared Spectroscopy: Fourier transforms infrared (FTIR) spectroscopy studies provide information on chemical functional groups of the sample. The IR spectral analysis was done using the press pellet technique using potassium bromide. The IR spectrum was recorded by using IR Prestige 21, Shimadzu. IR spectrometer in the region between 4,000 and 400 cm^{-1}.

Differential Scanning Calorimetry (DSC): Thermal properties were characterized using a differential scanning calorimeter (Hitachi STA 7300). The sample was sealed in an aluminum pan and heated from 25 to 725°C at a heating rate of 10°C/min in a nitrogen atmosphere. DSC is a method used to study the drug's thermal properties and physicochemical characteristics in the designed formulation.

The existence of any separate peak of components in the formulation DSC confirms that there is no interaction between the drug and polymer. On the contrary, the appearance of one or two new peaks or shift in their locations demonstrate the interaction between two components.

Powder X-ray Diffractometry: Powder X-ray diffractometry (XRD) studies were performed with the X-Pert pro, PAN analytical model X-ray diffractometer using Ni-filtered Cu-K(α) radiation, a voltage of 40 kV and current of 30 mA. The width of receiving slit is 0.3 mm. XRD is used for knowing a crystal's atomic and molecular formation, in which the crystalline atoms make a beam of dependent X-rays to diffract into many particular directions. The sample was analyzed over the 2θ range of 10-80°, scan speed of 4.0"°/min" with scan step and scan time of 0.020° and 0.3 sec respectively

Preparation of Mesalamine Loaded Microsponges [8-10]: Micro sponges were prepared by quasi-emulsion solvent diffusion method. Ethanol as the internal phase and 0.5% w/v polyvinyl alcohol in distilled water as the external phase was used to prepare microsponges. Initially required concentration of drug and polymer were dissolved in 5 ml of ethanol. Ethanolic solution of drug and polymer was added dropwise to 100 ml of external phase in a 250 mL beaker with continuous stirring using a mechanical stirrer (Remi motor 4000rpm) at a speed of 1000 rpm. Stirring was

continued for a period of 8 hrs. The formulated micro sponges were separated by filtration using Watsman filter paper of grade 42 and dried at 40°C for 12 hrs. Different batches are prepared as per **Table 1.** Process variables like stirrer speed, stirring time, and volume of the beaker are validated by making the initial trails, and finally, the conditions were optimized. Microsponges were equivalent to 10-12 doses of mesalamine prepared each time.

Evaluation of Microsponges [11-16]: The prepared microsponges were evaluated for drug content, percentage yield, entrapment efficiency, and surface morphology.

Determination of Percentage Yield: Percentage yield is the ratio of the initial mass of the drug and polymer to the mass of obtained microsponges. The following formula calculated the percentage yield:

Percentage yield (PY) = Practical mass of micro sponges / Theoretical mass of microsponges × 100

Drug Content and Entrapment Efficiency: The weighed amount of drug-loaded microsponges (100 mg) was kept in 100 mL pH 6.8 phosphate buffer for 12 h with continuous stirring. The samples were filtered using a Whatman filter of grade 42, and the samples were analyzed at 330 nm against blank using UV spectrophotometer (UV 1700, Shimadzu). Entrapment efficiency was also calculated by the same method using the following formula:

% Entrapment efficiency (EE) = Mass of drug in micro sponge / Mass of Microsponges × 100

***In-vitro* Drug Release Studies of Microsponges:** *In-vitro* release of mesalamine from the prepared microsponges was studied using USP XXIV type I basket dissolution rate test apparatus (Model: DISSO 8000, M/s. Labindia). Four dissolution media were used to mimic the stomach-to-colonic transit environment in the following manner.

0-2 hours	0.1N HCl (pH 1.2)
2-4 hours	pH 4.5 phosphate buffer
4-6 hours	pH 6.8 phosphate buffer
6-till the end of dissolution	pH 7.4 phosphate buffer

The volume of the dissolution medium was 900 ml and maintained at a temperature of 37±0.5ºC. The basket was rotated at 50 rpm. 5 ml aliquots were withdrawn using a syringe fitted with a pre-filter at appropriate time intervals and immediately replaced with 5 ml of fresh medium maintained at 37±0.5ºC. The buffers were replaced at the end of time as specified above. At the end of each time interval, the samples were analyzed for mesalamine content at 330 nm.

Drug Release Kinetics and Mechanisms: Analyzing the drug release mechanism from a pharmaceutical dosage form is an important but complicated process. The order of drug release was described by using zero order kinetics or first-order kinetics. The drug release mechanism was studied using Higuchi, erosion, and Peppas equations.

Zero order Release Kinetics: It defines a linear relationship between the fraction of drug released versus time. Eq. 3.1 describes zero-order kinetics.

$$Q = k_o t$$

Where, Q is the percentage drug release at time t and k_o is the zero-order release rate constant. A plot of the percentage drug release against time will be linear if the re-release obeys zero-order release kinetics.

First-Order Release Kinetics: Wagner, assuming that the exposed surface area of a tablet decreased exponentially with time during the dissolution process, suggested that drug release from most slow-release tablets could be described adequately by apparent first-order kinetics. Eq. 3.2 is used to describe first-order kinetics,

$$Log(1-Q) = -k_1 t$$

Where, Q is the percentage of drug released at the time (t) and k_1 is the first order release rate constant. A plot of the logarithm of the percentage of drug remaining against time will be linear if the release obeys first-order release kinetics.

Higuchi Equation defines a linear dependence of the active fraction released per unit of surface (Q) on the square root of time. The Higuchi model is explained by Eq. 3.3.

$$Q = k_H t^{1/2}$$

Where, Q is the percentage of drug released at time t, k_H is the Highuchi diffusion coefficient. A plot of the percentage of drug released against the square

root of time will be linear if the release obeys the Higuchi equation.

Erosion Equation: This equation defines the drug release based on tablet erosion alone. The equation for erosion is

$$Q = 1-(1-k_E t)^3$$

Where, Q is the percentage of drug released at time t, k_E is the Hixson and Crowell constant.

A plot between $[1-(1-Q^{1/3}]$ against time will be linear if the release obeys erosion equation.

Korsmeyer-Peppas Equation: Korsmeyer-Peppas support the drug release mechanisms for further judgment.

$$Q_t/Q_\infty = Kt^n$$

Where, Q_t/Q_∞ is the fraction of drug released at time t, K is the Korsmeyer-Peppas constant and 'n' is the release exponent.

According to Korsmeyer-Peppas equation, the release exponent 'n' value is used to characterize different release mechanisms for a dosage form with cylindrical shape, summarized below.

Diffusion exponent (n)	Drug release mechanisms
0.45	Fickian diffusion
0.45<n<0.89	Anomalous(non-Fickian) diffusion
0.89	Case II transport
n > 0.89	Super Case II transport

Evaluation of Micromeritic Properties [17-20]: The optimized microsponges (ES5, EL5, ERS5, RSPO5) were passed through sieve no. 18 (ASTM, mesh aperture size 1 mm) and were evaluated for micrometric properties like angle of repose, bulk density, tapped density, Carr's (compressibility) index, Hausner's ratio. All the determinations were performed in triplicate and average values are reported.

Angle of Repose: Angle of repose was determined by the fixed funnel and free-standing cone method. A funnel with the end of the stem cut perpendicular to its axis of symmetry was fixed at a given height (h) above the graph paper placed on a flat horizontal surface. The powder was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. The radius 'r' of the base of the pile was determined and the angle of response was calculated by following equation.

$$\text{Tan } \theta = h / r$$

Bulk Density: Bulk density of a powder is the ratio of the mass of the powder to the volume occupied by the loose powder bed. Microsponges equivalent to 10 g was accurately weighed and placed in 25 mL measuring cylinder without compaction and the volume occupied was measured and the following equation calculated the initial bulk density.

Bulk density = Mass of powder / Bulk volume of the powder

Tapped Density: Tapped density of a powder is the ratio of the mass of the powder to the volume occupied by the powder after a fixed number of taps. The tapped density of the powder represents its random dense packing.

Microsponges taken to determine bulk density were placed on to the tapped density tester (Model C-TDA2, Campbell Electronics, Mumbai, India) and subjected to USP-I method i.e., 300 drops per minute with a drop height of 14±2 mm for 500 tapping's. The volume (Vt) of the powder bed was measured after 500 tapping's. The tapping was repeated for additional 750 times and the volume was noted as (Vb). The difference between the two volumes was less than 2%; hence, Vb was considered a tapped volume. Tapped density was calculated by the following equation.

Tapped density = Mass of the powder / Tapped volume of the powder

Carr's (Compressibility) Index (CI): Carr's index of the powder was found by using the following equation.

CI = Tapped Density-Bulk density / Tapped density

Hausner's Ratio (HR): Hausner's ratio of a powder is expressed as a ratio between tapped density and bulk density.

HR = Tapped Density / Bulk Density

Preparation of Micro Sponge Core Tablets: Microsponges equivalent to a dose of 500 mg were compressed into core tablets by direct compression method. The formulae of the prepared core tablets

are shown in **Table 2.** All the ingredients were sufficient for a batch of 100 tablets were weighed and mixed in a polybag by geometric dilution method for 15 min. The final powder mixture was compressed into tablets using 12 mm round flat punches on a 12-station rotary tablet machine (Karnavati Engineering Ltd., India) using compression force sufficient for obtaining hardness in the range of 4-5 kg/cm^2.

Evaluation of Micro Sponge Core Tablets [21-23]: The prepared core tablets of microsponges were subjected to quality control tests such as uniformity of weight, hardness, thickness, friability test and *in-vitro* dissolution studies.

Uniformity of Weight: According to I.P, twenty tablets were selected randomly, and weighed together and individually to determine the uniformity of weight of tablets.

The average weight and % deviation of individual tablets from average weight was determined. Prepared core tablets (700 mg) comply with the test if not more than two of the individual weights deviate from the average weight by more than 5% and none deviate more than twice the 5%.

Hardness Test: Five tablets were selected at random, and the hardness of each tablet was measured on Monsanto hardness tester. It is expressed in kg/cm^2.

Friability Test: The friability test was carried out in Roche friabilator. 10 tablets were randomly selected and the initial weight (wo) was noted. They were placed in the rotating drum of the friabilator and subjected to 100 rotations to fall from the height of 6 inches at a rate of 25 rpm. After completion of rotations, the tablets were again weighed (w). Prepared tablets comply the test if the percentage of friability is within the Pharmacopoeias limit (not more than 1%).

Thickness Measurement: Five tablets were selected randomly from each formulation, and each formulation's average thickness was measured with Vernier calipers' help.

***In-vitro* Dissolution Studies:** *In-vitro* dissolution studies were done in 0.1N HCl (pH 1.2), pH 4.5 phosphate buffer, pH 6.8 phosphate buffer, pH 7.4 phosphate buffer per the procedure.

Preparation of Compression-Coated Tablets of Microsponges: All the mesalamine core tablets were subjected to compression coating to study their suitability for colon targeting. For achieving the targeting colon, it is proposed to achieve a lag time of 6 hrs for preventing mesalamine release in the stomach and small intestine. Cellulose acetate phthalate (CAP) was selected as a polymer for compression coating. To optimize the weight of CAP required for compression coating to achieve the desired lag time of 6 hrs, initial trials were conducted using placebo tablets of similar dimensions with similar tableting characteristics. The prepared placebo compression-coated tablets were tested for tableting characteristics and resistance to disintegration in the dissolution media (0.1N HCl during 0-2 hrs; 2-4 hrs. pH 4.5 phosphate buffer; 4-6 hrs. pH 6.8 phosphate buffer) used in dissolution studies up to 6 hours and finally the weight of CAP was optimized as 100 mg.

The Coating material was compressed around the core tablet using round flat punches with punch size 14 mm on a 12-station rotary tablet machine (Karnavati Engineering Ltd.) using compression force sufficient for obtaining hardness in the range of 5-6 kg/cm2. Compression-coated tablets were prepared as per the formulae given in **Table 3.** 50% of the coating material was placed in the die cavity, and the core tablet was placed in the center of the die, followed by the addition of the remainder of the coating material and tablet was compressed.

Evaluation of Compression-Coated Tablets of Microsponges [24-26]: The prepared compression-coated tablets were subjected to quality control tests such as uniformity of weight, hardness, thickness, friability test, and *in-vitro* dissolution studies.

Simulation of Colonic Environment for *In-vitro* Drug Release: Human colon represents a dynamic and ecologically diverse environment, comprising over 400 distinct species of bacteria with a population of 109 –1010 CFU/mL colonic contents. These bacteria produce a wide spectrum of reductive and hydrolytic enzymes responsible for many bio-relevant processes like carbohydrate and protein metabolism. Hence, to simulate the colonic milieu *in-vitro*, the methodology should be designed to involve the microbial of colon.

It is always advisable to test the dissolution of the drug from CTDDS in the presence of microbial flora of the large intestine. Hence, different dissolution media containing either colonic enzymes, rat cecal contents, human fecal contents, or probiotics media were reported earlier [9]. In the present investigation, a probiotic colonic dissolution medium was used to test microbial flora's influence on the drug release of the prepared mesalamine colon-targeted tablets by replacing the pH 7.4 phosphate buffer with probiotic media from 6 hrs to end of the dissolution under anaerobic conditions.

Preparation of Probiotic Culture Medium: 1000 mL of pH 7.4 phosphate buffer containing approximately 1010-1011 colony-forming units of *Bacillus clausii* was prepared by adding 3 mL of the commercially available product (Enterogermina: *Bacillus clausii* 2 billion spores per 5 mL oral suspension SANOFI Synthelabo, Bhiwandi, India) and was maintained under anaerobic conditions by continuously bubbling CO2 for 30 min.

***In-vitro* Dissolution Testing in Probiotic Culture Medium:** *In-vitro* release of mesalamine from the prepared compression-coated tablets was studied using USP XXIV type I basket dissolution rate test apparatus (Model: DISSO 8000, M/s. Labindia). As described in the sec. 2.5.3 the initial dissolution studies were carried out in 0.1N HCl (0-2 hrs), pH 4.5 phosphate buffer (2-4 hrs), pH 6.8 phosphate buffer (4-6 hrs) for compression-coated tablets. At the end of 6 hrs the medium was replaced with 900 ml of probiotic culture medium as prepared above and dissolution was continued under anaerobic conditions by continuous bubbling with CO_2 to mimic the colonic environment till the end of the dissolution. The rotation speed was 50 rpm, and the temperature was maintained at 37±0.5ºC. *In-vitro* dissolution studies were done in 0.1N HCl (pH 1.2), pH 4.5 phosphate buffer, pH 6.8 phosphate buffer, pH 7.4 phosphate buffer as per the procedure.

Pharmacodynamic Studies of Selected Mesalamine Compression Coated Tablets for Colon Targeting: Mesalamine is a drug whose in vivo performance is well established and hence, CT-ES5 compression coated mesalamine tablet, which extended the drug release throughout 24 hrs after lag time was tested for *in-vivo* performance in rats.

The study was carried out in accordance with the approval of the Institutional Animal Ethical Committee (Reg. No. 516/01/A/CPCSEA) of A.U. College of Pharmaceutical Sciences, Andhra University, Vishakhapatnam (India).

Use of Rat Model in the Study of Bioavailability: Rats can be easily bred, low cost, and easy to handle. Rats are a suitable model for inflammation studies; these show similarities concerning humans. These animals were suitable for pharmacodynamic studies. Because of these criteria, rats were selected for the study. In rats it is easy to induce IBD by using 2 ml acetic acid (4% v/v) as a colitis inducing agent.

Adult male Wistar rats (200-250 g) were purchased from Mahaveer Enterprises, Hyderabad. The animal room was maintained at 22°– 24°C with a 12-hour light/12-hour dark lighting regimen. Rats were fed with standard house chow and water ad libitum.

Rat Dose of Mesalamine [27, 28]: Rat dose of mesalamine was fixed based on the earlier reports as 25 mg/kg3,4. As 200-250 g of rats were used in this study, compression-coated mesalamine tablets were prepared from ES5 microsponges equivalent to 5 mg of mesalamine.

Preparation of Compression Coated Microsponges for Rat Administration (RCT-ES5): ES5 microsponges equivalent to 5 mg of mesalamine were compressed into a core tablet using multi-pin head punches (shown in **Fig. 1**). The prepared mini core tablets of 2 mm diameter were further compression coated with 10 mg of CAP to obtain compression coated tablet (RCT-ES5) having a diameter of 4 mm. The images of the prepared mini-core and compression-coated tablets are shown in **Fig. 2.**

Evaluation of Mini Tablets of Rats (RCT-ES5): The prepared tablets were evaluated for tableting characters as described in the sec. 2.5.3.

Pharmacodynamic Evaluation of Mesalamine RCT-ES5 [29]: The pharmacodynamic activity of the

prepared mesalamine RCT-ES5 was carried in rats induced with colonic inflammation by using 2 mL of 4% v/v acetic acid in 0.9% saline as colitis inducing agent [5].

Experimental Design: The rats in the weight range of 200-250 g were divided into four groups, each containing three rats.

Group I: Control group without disease and treatment.

Group II: Disease-induced rats treated with placebo tablets. (Disease control)

Group III: Disease-induced rats and treated with pure mesalamine drug (5 mg/rat, oral)

Group IV: Disease-induced rat treated with RCT-ES5

Induction of Colonic Inflammation to Rats: All animals (except group I) were fasted for three days before the study, with access to water *ad libitum* and after three days, the rats were anesthetized by an intra peritoneal injection of 1% sodium pentobarbital at a dose of 50 mg/kg. Anesthetized rats were kept in a supine trendelenburg position as shown in **Fig. 3**. A soft pediatric catheter size of 6F, 2 mm in diameter, was inserted through the rectum into the colon up to a distance of 8 cm and slowly infused 2 ml of 4% v/v acetic acid in 0.9% saline for 30 seconds. The supine trendelenburg position was maintained for another 30 seconds to prevent intra-colonic leakage and remove the catheter. The rats were kept in the cage for 24 hrs for induction of colitis. After 24 hrs of disease induction, group II was treated with placebo tablets, group III, group IV were treated with mesalamine pure drug and RCT-ES5, respectively. The rats were sacrificed by cerebral dislocation after 24 hrs of treatment.

Assessment of Inflammation: Each animal was dissected. The distal 10 cm portion of the colon was removed, cut longitudinally, cleaned with physiological saline to remove fecal residues, and kept in 10% formalin. The results were interpreted based on the macroscopic scoring and histological analysis of colon tissue samples.

Macroscopic Scoring [30]: The distal colon specimen soaked in formalin was immediately examined. Any visible damage was scored on a scale of 0-5 as given below, by two independent observers, compared with normal and disease control.

0: No damage

1: Localized hyperemia with no ulcer

2: Linear ulcers with no significant inflammation

3: Two or more sites of ulceration or inflammation

4: Two or more major sites of inflammation and ulceration at one or major sites of inflammation ulceration extending > 1 cm.

Histological Analysis [30]: The colon tissue samples in formalin were processed for microscopic examination. They were embedded in paraffin. Sections were cut at 5 μm thicknesses on a rotary microtome, mounted and stained with hematoxylin and eosin (H and E). These sections were evaluated for histological changes under light microscopy.

The mucosal damage assessment was done according to the following scale:

0: Intact epithelium, no leukocyte or hemorrhage

1: <25% disrupted epithelium focal leukocyte infiltrates and focal hemorrhage

2: 25% disrupted epithelium focal leukocyte infiltrates and focal hemorrhage

3: 50% disrupted epithelium widespread leukocytes and hemorrhage

4: >50% disrupted epithelium extensive leukocyte infiltration and hemorrhage

RESULTS & DISCUSSION: Microsponges were prepared in four different batches by quasi-emulsion solvent diffusion technique using four different polymers with five different drugs to polymer ratios of 1:1, 2:1, 3:1, 4:1, and 5:1 using similar process variables. Initially, the microsponges were prepared with an increased concentration ratio of polymer concerning the drug. The yield of microsponges was very low, and the microsponges were less porous. This may be due to the highly viscous nature of the internal phase of the polymer solution. Hence, microsponges were formulated with a drug-to-polymer ratio of 1:1 to

5:1. Formulated microsponges are shown in **Fig. 1**. They were found to be flakes after filtration and were found to be in the form of flakes after filtration powdered gently in a glass mortar and passed through sieve no. ASTM 18 mesh aperture size 1 mm as per IS and stored in a glass bottle. These formulations were evaluated for drug content, percentage yield, entrapment efficiency, and *in vitro* drug release kinetics.

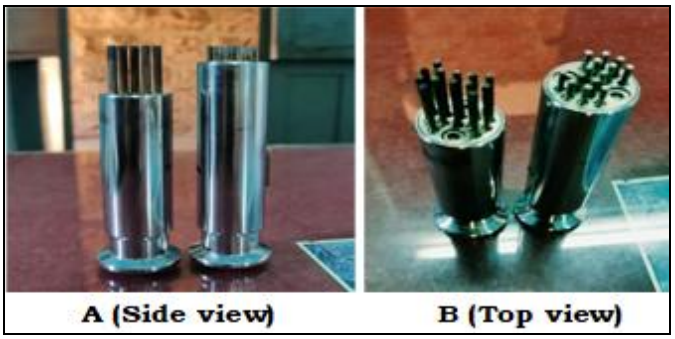


FIG. 1: MINI PUNCHES WITH 2MM DIAMETER MINI PROJECTIONS

Drug Content, Percentage Yield, Entrapment Efficiency: The percentage drug content, percentage yield, and entrapment efficiency for all formulations were calculated, and results are given in **Table 1**.

TABLE 1: FORMULAE OF PREPARED MESALAMINE MICROSPONGES

Mesalamine – 800 mg, Ethanol 95% v/v – 5 Ml, 0.5%w/v PVA - 100 mL	
Ratios of drug to polymer	**Polymers used (mg) eudragit S100**
1	800 (1:1)
2	400 (2:1)
3	267 (3:1)
4	200 (4:1)
5	160 (5:1)

Note: Formulations prepared with eudragit S100 named as ES1-ES5; Batch size: Equivalent to 10-12 doses of mesalamine

The percentage yield values were increasing with a decrease in polymer concentration. Percentage yield values for 1:1 to 1:5 ratios ranged between 54±0.23% to 94±0.13%. The drug content values were found to be in the range of 85-115% specifications, varying between 86.04±0.14% and 100±0.92%. Entrapment efficiency values for different formulations were in between the range of 81±0.32% to 96±0.16%. Entrapment efficiency also increased with a decrease in polymer concentration.

***In-vitro* Dissolution Studies:** *In-vitro* dissolution studies were carried out for microsponge formulations prepared with eudragit S 100. The mean percent of mesalamine released at different time intervals was calculated, and the results are shown in **Table 2,** and the drug release profiles are shown in **Fig. 2.**

FIG. 2: MINI CORE TABLETS, COMPRESSION COATED TABLETS (RCT-ES5)

TABLE 2: FORMULAE OF MICROSPONGE CORE TABLETS

Ingredients per tablet (mg)	T-ES5
Microsponge formulation equivalent to 500 mg of mesalamine	**625**
Lactose	67
Magnesium stearate	8
Total weight	700

An inverse relationship was observed between drug release and polymer concentration. ES5 with 1:5 ratio of drug-eudragit S100 showed 100.03±1.07% release in 12 hrs. With the increase in polymer concentration i.e., 1:4, 1:3, 1:2 and 1:1, the drug release was decreased and extended beyond 12 hrs. ES1 could release only 72% drug during 12 hours, indicating the influence of polymer concentration on drug release. As the drug release was extending beyond 12 hrs with increased polymer concentration, ES1 was considered for further study as complete drug release was obtained in 12 hrs. The rate of drug release from microsponges followed a biphasic pattern. The drug release followed first-order kinetics from 0 to 4 hrs and from 4 to the remaining period zero order kinetics was followed.

This may be due to the initial swelling of the polymer and hydration at the time the surface-adhered drug was released. This release mechanism can be explained as a result of drug diffusion through the microsponge formulation's porous polymeric surface. Drug release studies clearly

indicated slow and sustained mesalamine release from all the prepared microsponges. As the concentration of polymer is reduced, the drug release also slows down; it may be due to the highly porous nature of microsponge.

Drug Release Kinetics: The correlation coefficient (r) values of zero-order and first-order kinetics of all formulations are shown in **Table 3**. From the dissolution profile data, it was found that all the formulations showed biphasic drug releases. The drug release data was divided into phases: phase one from 0 to 4 hrs and phase two from 4 hrs till the end of dissolution. The correlation coefficient values of the first-order regression line were higher than the zero-order line for the first phase from 0-4 hrs indicating the drug release by the first-order process.

During the initial stages of dissolution, the adhered drug on the surface occurred and hence, the release followed first-order kinetics. The polymer was swollen due to contact with the dissolution medium and after complete swelling of the polymer, there is a change in the pattern of drug release and this resulted in the second phase in which the zero order correlation coefficients were higher compared to the first order indicating that the release followed zero order kinetics.

TABLE 3: FORMULAE OF COMPRESSION-COATED TABLETS

Ingredients (per one tablet)	CT-ES5
Core tablet (mg)	700(T-ES5)
CAP (mg)	100
Total weight (mg)	800

After the release of the surface-adhered drug, the release of entrapped drug starts occurring in a controlled way following zero-order release.

Drug release studies clearly indicated slow and sustained mesalamine release from all the prepared microsponges. As the concentration of polymer is reduced, the drug release also slows down, possibly due to microsponges' high porous nature.

Drug Release Mechanism: The drug release mechanism was determined by fitting dissolution data to the linear regression plots for dissolution profiles.

Higuchi and erosion plots were drawn and correlation coefficients of the release mechanism of all formulations are shown in **Table 4**. Correlation coefficients of Higuchi plots of all formulations were in between 0.9061 to 0.9983, and hence the mechanism of drug release followed Higuchi diffusion.

TABLE 4: EVALUATION PARAMETERS OF MESALAMINE MICROSPONGES

Formulation	Percentage yield (%±s.d.)	Drug content (%±s.d.)	Entrapment efficiency (%±s.d.)
ES1	54±0.23	86.04±0.14	84±0.23
ES2	58±0.45	89.53±0.23	88±0.87
ES3	79±0.04	90.35±0.42	89±0.20
ES4	85±0.26	91.24±0.39	85±0.30
ES5	94±0.13	100.12±1.31	96±0.16

The data was further subjected to Korsmeyer equation for differentiating between Fickian and non-Fickian diffusion and the exponent n was calculated using the slope of the straight line and the values of ‘n’ are shown in **Table 4.** Exponent ‘n’ values for microsponges prepared with eudragit S100 were above 0.45 and hence they followed non-Fickian diffusion.

All the formulations with eudragit polymers showed drug release by the mechanism of diffusion due to porous polymeric diffusive barrier. After 2 to 4 hrs the thickness of the barrier was increased due to the swelling of the polymer and drug release controlled by zero order kinetics.

Morphology: The morphology and surface characteristics of the selected microsponges were studied using scanning electron microscopy (SEM). All the samples were coated with gold–palladium alloy under vacuum.

Coated samples were then examined using JSM-6610LV SEM analyzer. Optimized samples were evaluated for surface morphology and SEM images revealed that all formulations were porous, spherical, sponge like texture with void spaces. SEM images of the selected drug loaded microsponges are shown in **Fig. 3.** SEM images of drug loaded sponges were compared with the placebo microsponges prepared with the respective

eudragits for examining the presence of void spaces and drug loading.

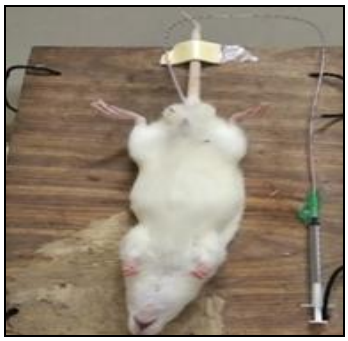


FIG. 3: TRENDELENBURG POSITION OF RATE DURING INDUCTION OF COLITIS

(A) NORMAL PHOTOGRAPH (B) MAGNIFIED PORTION

FIG. 4: PHOTOGRAPH OF FORMULATEFD MICROSPONGEGE OF MESA LAMINE

It was observed that the void spaces and porous structure of the empty microsponge is occupied with the drug in drug-loaded microsponges, indicating the clear entrapment of drug by microsponges. Comparative SEM images of placebo microsponge prepared with eudragit S100 and the respective microsponge ES5 are shown in **Fig. 4.**

Evaluation of Micromeritic Properties of Microsponges: The micrometric properties such as angle of repose bulk density, tapped density, compressibility index and Hausner's ratio depend mainly on particle size distribution, particle shape and tendency of the particles to adhere together. These micrometric properties are often referred as the derived properties of powders. They play an important role in the hopper filling, since the powder mass's flow characteristics are very important. The results of the micrometric properties of the microsponge are shown in **Table 5.** Angle of repose value below 25° indicates excellent flow. The angle of repose values of all the microsponges are below 25° indicating excellent flow properties.

TABLE 5: CUMULATIVE PERCENT OF MESALAMINE RELEASED VS. TIME FROM EUDRAGIT S100 MICROSPONGES

D.M.	Time (hrs)	Mean percent (mean±s.d., n=3) mesalamine released				
		ES1	ES2	ES3	ES4	ES5
pH 1.2	1	20.86±0.45	20.17±0.27	20.03±0.37	20.1±0.28	27.97±0.24
	2	28.73±0.61	28.4±0.48	33.18±0.29	33.09±0.75	36.03±0.46
pH 4.5	3	39.46±1.24	38.62±0.46	42.05±0.37	49.43±1.29	50.68±0.59
	4	46.73±1.56	44.96±0.65	51.85±0.53	53.25±1.08	59.70±1.18
pH 6.8	5	49.64±1.66	46.86±0.15	52.18±0.33	61.20±0.09	64.22±0.05
	6	51.19±0.94	50.75±0.84	53.21±0.78	68.72±1.73	69.59±0.37
pH 7.4	7	54.31±0.07	53.20±1.41	63.54±1.21	72.67±1.48	75.61±1.58
	8	58.34±1.27	57.10±1.57	67.53±1.32	75.67±1.54	80.61±1.32
	9	66.65±1.73	65.25±1.44	72.84±1.41	82.19±1.67	84.85±1.06
	12	72.21±1.86	78.36±0.72	80.78±1.40	98.76±1.26	100.03±1.07

CI value can reflect the ease of powder consolidation. A high CI value means poor flow. CI values up to 15% exhibits excellent flow properties, whereas CI >15% and <25% indicates good flow properties and more than 25% indicates poor flow properties.

The CI values of the microsponges were found to be in the range of 12.18-15.27%. The CI values of EL-5 and ERS-5 values are slightly more than 15% indicating good flow rather than excellent. HR value reflects inter particulate friction.

A high HR value means poor flow. HR value of less than 1.25 indicates a good flow property and more than 1.25 indicates poor flow property. All the HR values of the microsponges were found to be in the range of 1.14-1.18, indicating good flow.

Hence, by comparing the three properties *viz*. angle of repose, CI and HR, it can be concluded that the prepared microsponges are suitable for direct compression and hence, core tablets were prepared by using direct compression technique.

Uniformity of Weight, Hardness, Thickness and Friability: The core tablets were evaluated for weight variation, hardness, friability and thickness and the results are given in **Table 6**. The maximum percentage deviation for weight was found to be 2.65%, which is well below the specified limit of 5%. Hence, all the prepared tablets of mesalamine complied with the compendial standards for uniformity of weight. The hardness for all the formulations was found to be in the range of 4.1-4.5 kg/cm^2. The thickness of tablets ranged between 4.0 to 4.2 mm. The friability values of all formulations were found to be between 0.25 to 0.38%, indicating that the test complies with the official compendia test for tablets as per IP. Thus, the core tablets of mesalamine were regarded as good quality, fulfilling the official requirements of tablets.

TABLE 6: CORRELATION COEFFICIENTS (R) VALUES OF MESALAMINE RELEASE KINETICS FROM MICROSPONGES

Formulation Code	Zero order				First order			
	0-4hrs		Beyond 4 hrs		0-4hrs		Beyond 4 hrs	
	k_0 (mg.hr^{-1})	r	k_0 (mg.hr^{-1})	r	k_1(hr^1)	r	k_1(hr^1)	r
ES1	11.15	0.878	3.44	0.982	0.091	0.992	0.0012	0.918
ES2	10.67	0.873	4.38	0.989	0.924	0.989	0.0016	0.912
ES3	12.51	0.883	4.03	0.973	0.101	0.997	0.0029	0.905
ES4	13.61	0.879	5.46	0.995	0.126	0.988	0.021	0.908
ES5	14.21	0.873	7.32	0.984	0.125	0.992	0.0017	0.918

***In-vitro* Dissolution Studies of Microsponge Core Tablet:** The cumulative percent of mesalamine released at different time intervals from core tablets was calculated. The results are shown in **Table 7** and **Fig. 5.** The drug release from all the core tablets prepared with microsponges indicated 100% drug release. Core tablets prepared with T-ES5 (eudragit S100) released 100% drug in 12 hrs. The morphology of core tablets of microsponges T-ES5 is shown in **Fig. 6** in comparison with microsponges ES5.

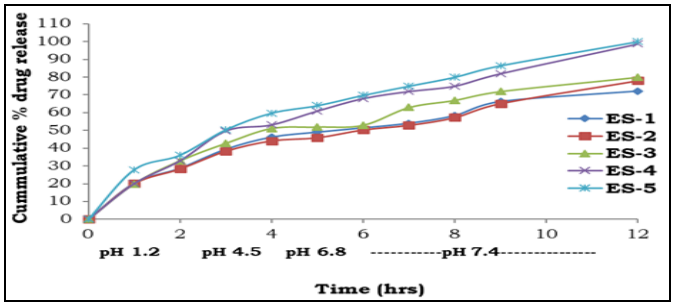


FIG. 5: DISSOLUTION PROFILES OF MESAMINE-EUDRAGIT S100 MICROSPARE

The void spaces were reduced to some extent, and the porous surface was also slightly altered compared to microsponges, which may be due to compression.

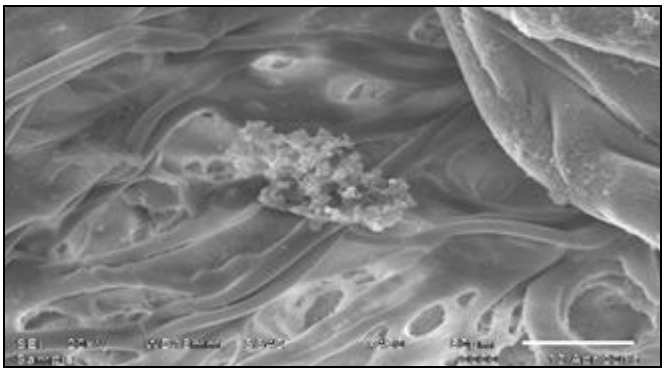


FIG. 6: SEM IMAGES OF ES5 SPONGES

However, there was no significant difference in the drug release pattern. Hence, it can be concluded that the compression of microsponges did not alter the porosity and void space to a great extent. Hence, it was concluded that the compression of microsponges into tablets did not influence drug release. As all the prepared core tablets could extend the drug release throughout 12-15 hrs, these core tablets were further subjected to compression coating for colon targeting.

TABLE 7: CORRELATION COEFFICIENT (R) AND PEPPAS EXPONENT (N) VALUES OF MESALAMINE RELEASE MECHANISM FROM MICROSPONGES

Formulation	Higuchi r	Erosion r	Peppas r	n value
ES1	0.9952	0.8681	0.9921	0.5074
ES2	0.9971	0.8894	0.9938	0.5229
ES3	0.9945	0.8915	0.9969	0.5391
ES4	0.9948	0.8962	0.9910	0.5183
ES5	0.9983	0.8884	0.9955	0.5239

***In-vitro* Dissolution Studies of Compression Coated Tablets of Mesalamine:** *In-vitro* dissolution studies were carried for compression-coated tablets of mesalamine. The results are shown in **Table 8** and **Fig. 7.** During the first 6 hours of dissolution the drug release from all the formulations was below 1% and thus the objective of the desired lag time was achieved with all the formulations. The drug release started at 6th hr of administration and showed a maximum drug release of 100.99% throughout 32-36 hrs. Compression coating produced a hard surface and compact structure due to which the drug release was delayed over 26-30 hrs after lag time of 6 hrs. The objective of the present study is to localize the drug in the colon for a prolonged period for treating IBD. Hence, these formulations extended the drug release for a prolonged period.

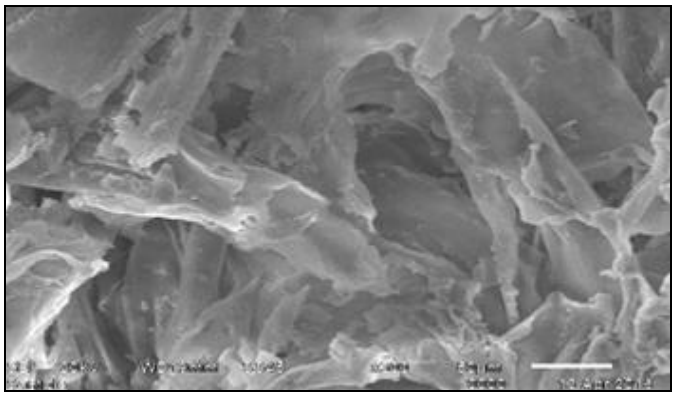


FIG. 7: SEM IMAGES OF EMPTY SPONGES

***In-vitro* Dissolution Studies of Compression Coated Tablets of Mesalamine in Probiotic Culture Medium:** The cumulative percent of mesalamine released at different time intervals in probiotic culture medium was calculated and the results are shown in **Table 9** and the drug release profiles are shown in **Fig. 8.**

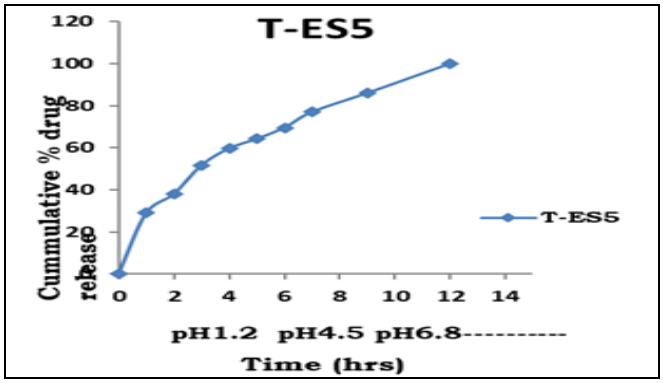


FIG. 8: DISSOLUTION PROFILES OF MESALAMINE MICROSPHERE CORE TABLETS

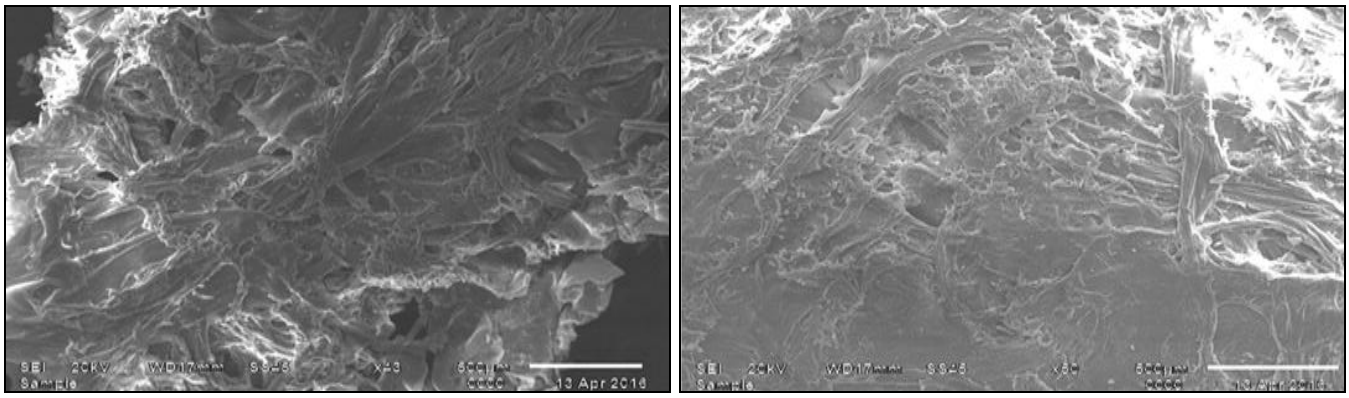
TABLE 8: MICROMETRIC PROPERTIES OF MICROSPONGES

Formulation	Angle of repose (°)	Bulk density (g/cc)	Tapped density (g/cc)	CI (%)	Hausner's ratio
ES5	19.45	0.71	0.81	12.18	1.14

The dissolution studies showed that all the compression-coated formulations maintained a lag time of 6 hrs. Tablet CT-ES5 prepared with eudragit S100 showed complete drug release in probiotic culture medium throughout 30 hrs, including lag time. The dissolution rate was increased in the probiotic culture medium for all the formulations, which may be attributed to the effect of microflora. Hence, it showed a faster drug release than the normal dissolution medium. The dissolution data of compression-coated tablets were analyzed for drug release kinetics and mechanisms from the commencement of drug release, i.e., from 6 hours.

Morphology: The morphology and surface characteristics of all the compression coated tablets of microsponges were studied using scanning

electron microscopy (SEM) as shown **Fig. 9**. Compared with microsponges **Fig. 9** the porous surface was completely covered by the compression coating polymer CAP.



(A) ES5 MICROSPONGES **(B) CORE TABLETS OF MICROSPONGES T-ES5**

FIG. 9: COMPARATIVE SEM IMAGES OF ES5 AND T-ES5

Results of Drug-excipient Compatibility Studies: The excipients are chosen, and their concentration and characteristics can influence the final drug product. By the literature on mesalamine with polymers, mesalamine did not interact with the polymers in the formulations.

According to ICH guidelines, drug-excipient compatibility studies are normally carried out for a physical mixture of drugs and selected excipient. However, drug-excipient incompatibility is possible due to other factors (processing variables) and during compression due to heat rather than a physical mixture. Hence, compatibility studies were carried out for microsponges and compressed tablets instead of physical mixtures. The techniques employed in the present work to study the drug-polymer interactions are Fourier transform infrared spectroscopy, differential scanning calorimetry and X-ray diffraction studies. These studies were carried for pure drug mesalamine, eudragit S100 and microsponge formulation ES5 and the corresponding compression-coated tablet, CT-ES5.

TABLE 9: TABLETTING CHARACTERISTICS OF CORE TABLETS OF MICROSPONGE

Formulation	Uniformity of weight[a] (mg)	Hardness[b] (kg/cm^2)	Thickness[b] (mm)	Friability[c] (%)
T-ES5	700±2.39	4.2±0.16	4.0±0.02	0.25

a: mean±%deviation, n=20; b: mean±s.d., n=5; c: n=10

Infrared Spectroscopy (FTIR) Studies: The FTIR spectra are shown in **Fig. 9**. According to spectra bands of mesalamine (3089.15, 3050.57, 1610.57, 1454.51, 1358.77, 1136.80, 1136.21-1267.32, 685.41-816.40), can be found in the bands of microsponge formulation ES5, compressed tablet formulation CT-ES5 (3489.15, 3200-3100, 3050.57, 1610.57, 1454.51, 1348.77, 1131.80, 1189.21-1263.32 and 685.41-810.40 cm-1). Hence, the results of FTIR studies affirm the presence of unmodified mesalamine in the optimized formulation. All the formulations clearly showed retention of characteristic bands of the drug; hence, no chemical interaction or complexation occurred between mesalamine and polymers.

Differential Scanning Calorimetry (DSC): The DSC thermograms of pure drug mesalamine, eudragit S100, and selected formulations (ES5 and CT-ES5) are shown in **Fig. 10**. DSC thermogram of mesalamine showed a sharp endothermic melting peak at 286°C, this was in correlation with the melting point of the drug. This sharp endothermic peak represents its crystalline nature.

The polymer eudragit S100 showed an endothermic peak at 110°C. Formulation ES5 showed endothermic peaks at 110°C and 286°C, indicating no interaction between the drug and polymer during manufacturing. The endothermic peaks at 286°C showed mesalamine, and at 110°C, represented eudragit S100. The compression-coated tablets of ES5 also exhibited an endothermic peak at 287°C. However, the peak observed was broader and comparatively not sharp. This may be due to the effect of compression coating by CAP.

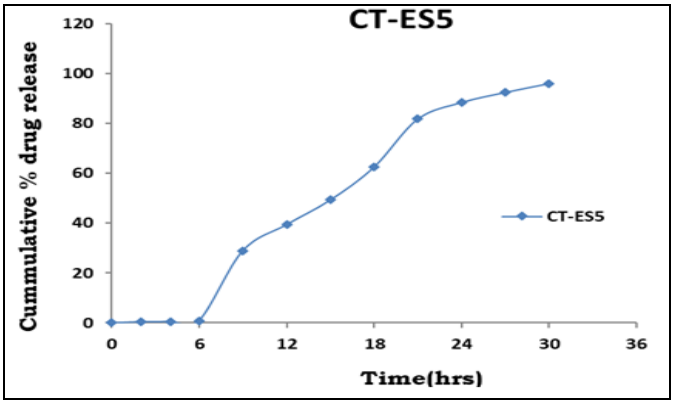


FIG. 10: DISSOLUTION PROFILES OF MESALAMINE COMPRESSION-COATED TABLETS

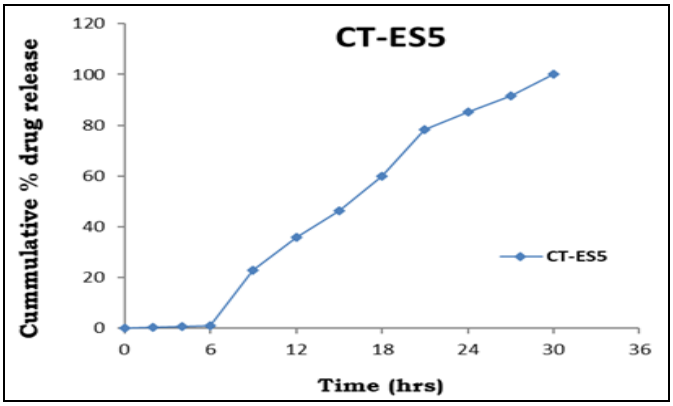


FIG. 11: DISSOLUTION PROFILES OF MESALAMINE COMPRESSION-COATED TABLETS IN PROBIOTIC CULTURE MEDIUM

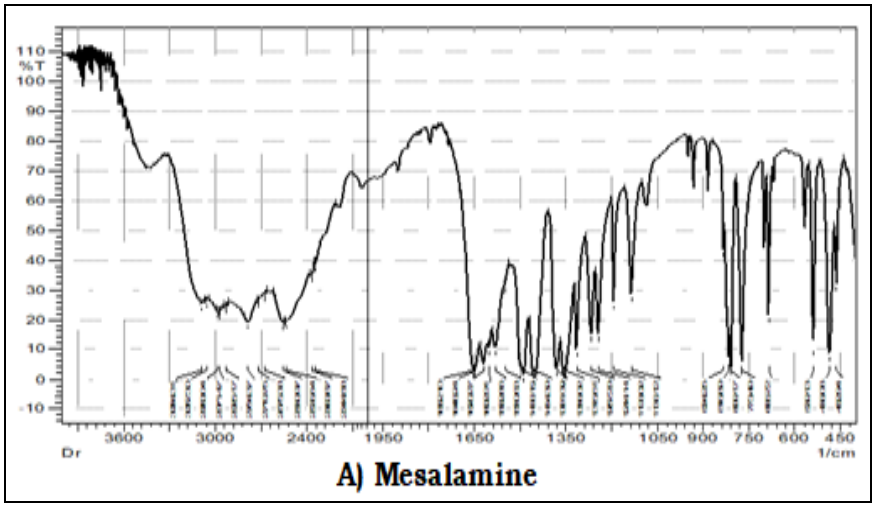
XRD Studies: The X-ray diffractograms of pure drug mesalamine, eudragit S100, ES5 and T-ES5) are shown in **Fig. 11**. The X-ray diffractogram of mesalamine showed sharp diffraction peaks at 15.5483°, 16.9634°, 24.6109°, 25.8718°, 27.5344°, 28.6345°, 30.916°, 37.0611°, 38.7710°.40.776° and 43.930° 2θ indicating the crystallinity of the drug. Pure polymer eudragit S100 showed broad peaks at 16.70708°, 38.132770° 2θ.

This indicated that the polymer selected in the formulation was not crystalline. In formulation ES5, the diffraction peaks at 15.405°, 16.8169°, 22.8558°, 24.495°, 25.779°, 27.4467°, 28.5359°, 36.9958°, 40.6818°, 43.8328°, 48.4899° 51.3206° and 55.0821° 2θ were observed coinciding with pure drug. However, the intensity was reduced, possibly due to the polymer's dilution effect. Similarly, the compression-coated microsponge core tablet also exhibited a peak with less intensity. These results indicated no change in the crystallinity of the drug.

Results of Evaluation of Mini Tablets of Rats: Results for weight variation, hardness, thickness, and drug content of RCT-ES5 mini tablets are given in **Table 10**. All the tableting parameters were within limits.

In-vitro dissolution studies were carried out for RCT-ES5 under similar conditions using 0.1N HCl for 2 hrs, pH 4.5 phosphate buffer for 2 hrs, pH 6.8 phosphate buffer for 2 hrs, and probiotic culture medium for the remaining period.

Dissolution profiles in the probiotic culture medium of CT-ES5 and RCT-ES5 are given in **Table 11** and **Fig. 12**. Dissolution profiles of RCT-ES5, and CT-ES5 were super imposable and indicated that both the tablet formulations were similar in the release profiles. Hence, RCT-ES5 was tested in rats for pharmacodynamic activities.



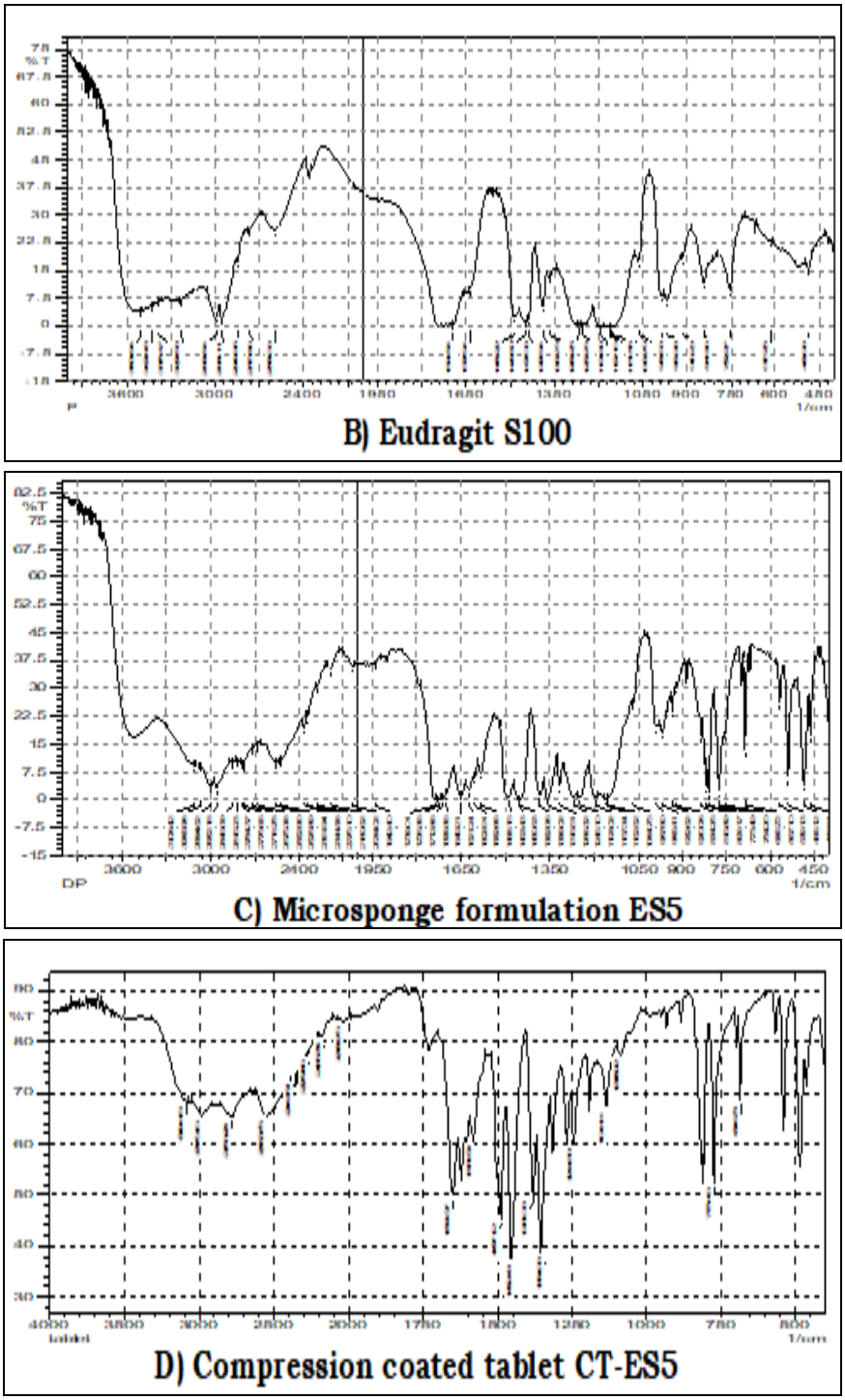


FIG. 12: FTIR SPECTRA

TABLE 10: CUMULATIVE PERCENT OF MESALAMINE RELEASED VS. TIME FROM MICROSPONGE CORE TABLETS

D.M.	Time (hrs)	T-ES5
pH 1.2	1	28.97±0.24
	2	38.03±0.46
pH 4.5	3	51.68±0.59
	4	59.70±1.18
pH 6.8	5	64.22±0.05
	6	69.59±0.37
	7	77.61±1.58
pH7.4	9	86.85±1.06
	12	100.01±1.07

TABLE 11: CUMULATIVE PERCENT OF MESALAMINE RELEASED VS. TIME FROM COMPRESSION-COATED TABLETS

D.M.	Time (hrs)	Mean percent (mean±s.d., n=3) mesalamine released
		CT-ES5
pH 1.2	2	0.49±0.14
pH 4.5	4	0.69±0.14
pH 6.8	6	0.914±0.04
pH 7.4	9	28.95±1.03
	12	39.46±0.14
	15	49.31±0.09
	18	62.49±1.09
	21	81.78±0.02
	24	88.46±1.86
	27	92.52±0.09
	30	96.05±0.03
	32	100.01±0.03

Pharmacodynamic Evaluation: Pharmacodynamic parameters were evaluated by macroscopic scoring and histopathology.

Macroscopic Scoring: Macroscopic study results are shown in **Fig. 13** and **14**.

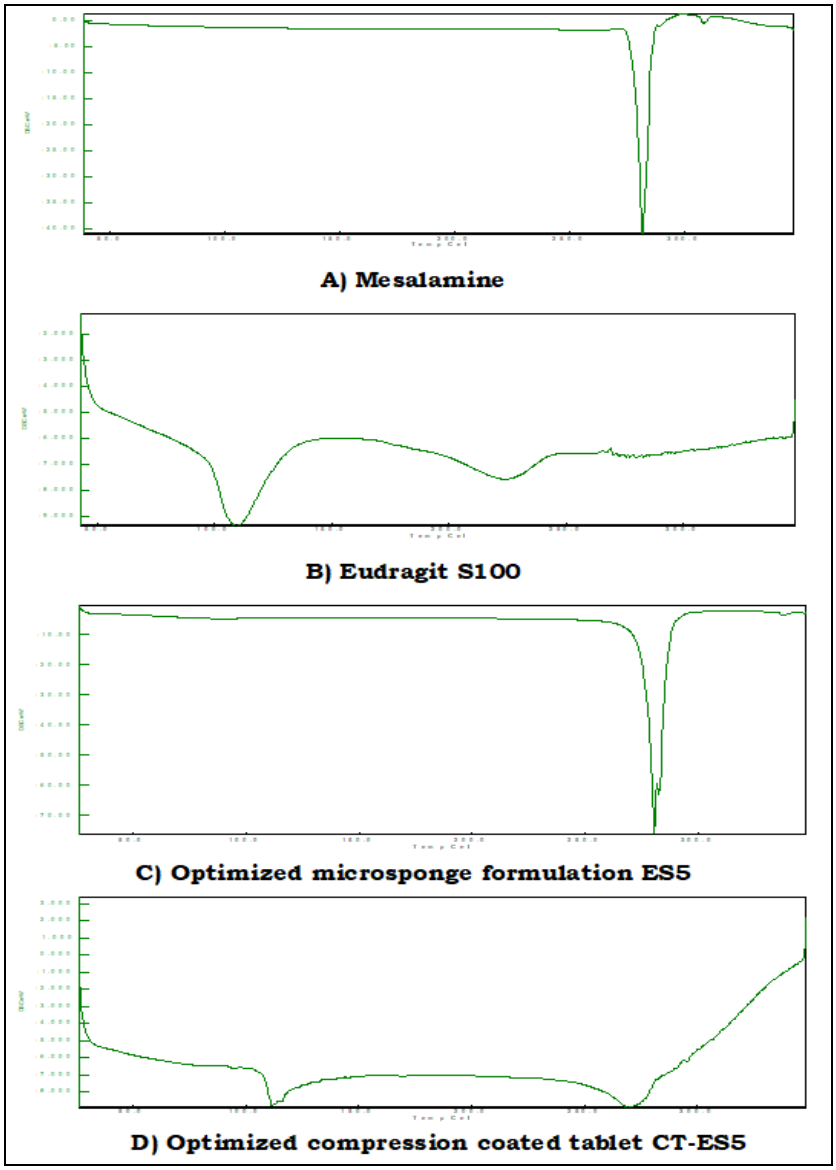


FIG. 13: DSC THERMOGRAMS

There was no damage or inflammation in control group I, and a score of '0' was observed. Disease control group II showed linear ulcers with inflammation, which confirmed the induction of the disease condition by using acetic acid, and the damage score was '4' for all the rats. In pure drug-treated group III, inflammation was reduced, and the damage score was '3'. The inflammation score was observed to be '1' for group 4 treated with optimized formulation, RCT-ES5. The results showed that compression-coated mesalamine microsponges effectively reduced the inflammation of colon tissue compared with pure drug-treated group.

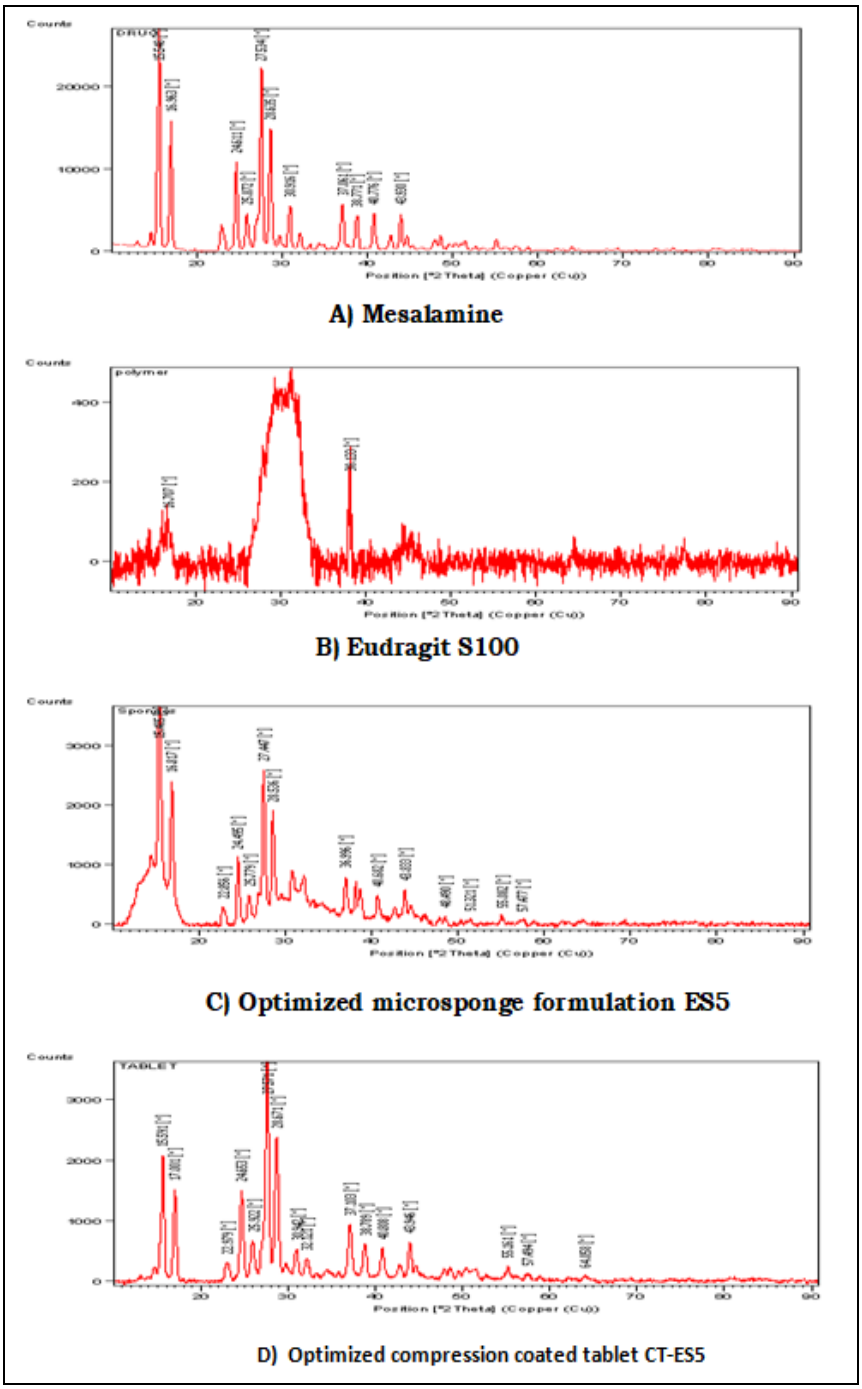


FIG. 14: X-RAY DIFFRACTOGRAMS

Histopathology: The histopathology results for experimental rats are presented in **Fig. 15** and **16**. Hematoxylin and eosin staining and light microscopic examination of colon tissue sections from control group rats revealed intact colon structure with no epithelial infiltration. Tissue sections from disease-induced rat groups revealed disruption of the epithelial lining and marked

infiltration of inflammatory tissue compared with the control group; the disruption score was '4'. Tissue sections from pure mesalamine-treated rats and optimized formulation (RCT-ES5) treated rats markedly reduced the induced disease. The epithelial infiltration score values for RCT-ES5 showed '1' compared with the pure drug score '2'.

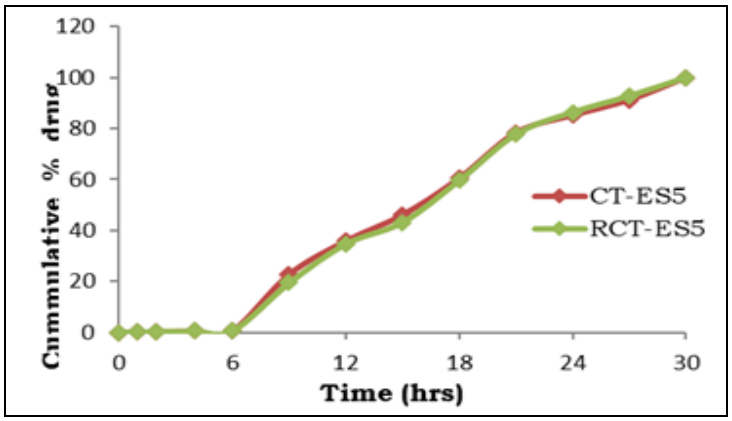
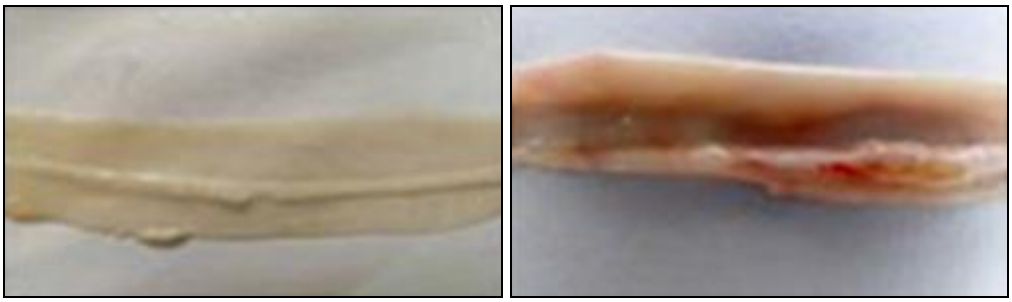


FIG. 15: DISSOLUTION PROFILE OF CT-ES5 AND RCT-ES5



(A) NORMAL **(B) DISEASE INDUCED**

(C) PURE DRUG TREATED **(D) RCT-ES5**

FIG. 16: PHOTOGRAPHS OF THE COLON FROM RAT

TABLE 12: CUMULATIVE PERCENT OF MESALAMINE RELEASED VS. TIME FROM COMPRESSION-COATED TABLETS IN PROBIOTIC CULTURE MEDIUM

D.M.	Time (hrs)	CT-ES5
Probiotic culture medium (pH 1.2, pH 4.5, pH 6.8)	2	0.36±0.14
	4	0.562±0.46
	6	0.862±0.24
	9	22.95±1.26
	12	35.99±0.94
	15	46.22±0.09
	18	60.59±1.07
	21	78.42±1.02
	24	85.21±1.86
	27	91.252±0.09
	30	100.01±0.1
	32	-
	34	-

TABLE 13: TABLETING CHARACTERISTICS OF MESALAMINE

Formulation	Uniformity of weight[a] (mg)	Hardness[b] (kg/cm^2)	Thickness[b] (mm)
CT-ES5	800±1.6334	4 – 5	5.1±0.03
RCT-ES5	16.5±1.05	2.5-3.5	1.1±0.02

a: mean±%deviation, n=20; b: mean, n=5;

TABLE 14: CUMMULATIVE PERCENT OF MESALAMINE RELEASED FROM CTES5 AND RCT-ES5

D.M.	Time (hrs)	Cummulative percent (mean±s.d., n=3) Mesalamine released	
		CT-ES5	RCT-ES5
Probiotic culture medium	pH 1.2 1	0.15±0.09	0.16±0.71
	2	0.36±0.14	0.29±0.51
	pH 4.5 4	0.52±0.14	0.49±0.51
	pH 6.8 6	0.862±0.24	0.612±0.24
	9	22.95±1.26	19.45±0.32
	12	35.99±0.94	34.73±0.75
	15	46.22±0.09	43.18±1.43
	18	60.59±1.07	59.69±0.96
	21	78.42±1.02	77.74±1.41
	24	85.21±1.86	86.36±1.22
	27	91.252±0.09	92.76±1.84
	30	100.01±0.03	100±0.67

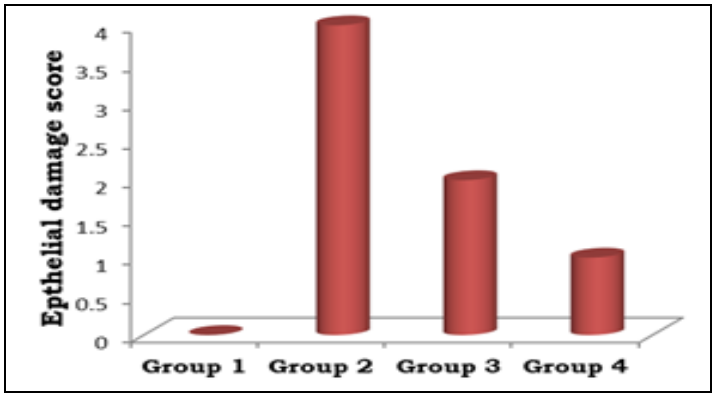
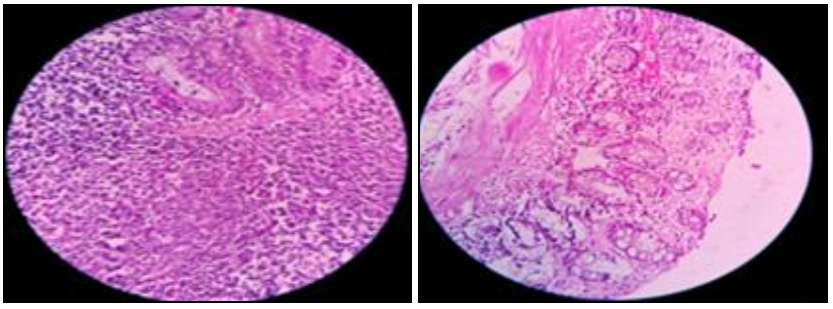
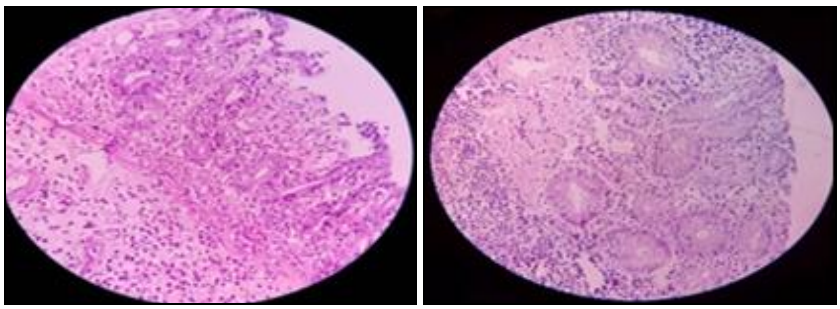


FIG. 17: BAR DIAGRAMS OF HISTOPATHOLOGY STUDY IN RAT



(A) CONTROL **(B) DISEASE**



(C) PURE DRUG TREATED **(D) RCT-ES5 TREATED**

FIG. 18: HISTOPATHOLOGY IMAGES OF RAT COLON

CONCLUSION: Mesalamine microsponges were prepared with different drug-polymer ratios using eudragit S 100. Initial trials were carried out for the preparation of microsponges. Increasing the polymer concentration resulted in lower yields of microsponges with less porous structure. Lump formation was also observed. Hence, drug concentration was increased compared to polymer, resulting in porous microsponges with good yield. Drug entrapment efficiency was also increased. The drug release from all the prepared microsponges was extended from 12 hrs and above. The drug release kinetics followed biphasic pattern with first order during 0-4 hrs and later with zero order kinetics. The mechanism of drug release was predominantly found to be Highuchi non-Fickian diffusion. Based on the % yield, entrapment efficiency, drug content, and complete drug release (12-15 hrs), ES5 is considered an optimized formulation.

A comparison of SEM images of empty microsponges with drug-loaded microsponges revealed the entrapment of drugs effectively in the microsponges. Selected microsponges were compressed into tablets and coated with the compression coating method. Microsponges exhibited good flow characteristics, so they were compressed into tablets by direct compression. Prepared core tablets confirmed all the tableting characteristics. The drug release from the core tablets of microsponges was extended from 12-15 hrs. The compression of the microsponges did not alter the release of mesalamine from core tablets. The drug release followed zero order kinetics as that of microsponges after 4 hrs with a non-Fickian diffusion mechanism. The core tablets were further subjected to compression coating using CAP as rate retarding polymer for achieving the desired lag time of 6 hrs which was optimized initially. The compression-coated microsponge core tablets confirmed to the requirements of tableting characteristics. There was no drug release during the first 6 hours of dissolution, confirming the suitability of these compression-coated microsponge tablets for colon targeting. The drug release from the compression-coated microsponge tablets was extended for a period ranging between 32-36 hrs again based on the nature of eudragit. The extension of drug release may be attributed to the compression coating with CAP. In a probiotic dissolution medium, these compression-coated microsponge tablets also showed extended drug release with a lag time of 6 hrs. However, compared to ordinary dissolution, the release was completed faster, i.e., 100% drug release was completed throughout 30-34. The increase in drug release in the probiotic medium may be due to the presence of microflora. The extended drug release for 30-36 indicated the suitability of these compression-coated microsponges for localization of drug in the colon for prolonged periods. This compression-coated tablet followed zero-order kinetics with non-Fickian diffusion in both media. Among all the compression-coated microsponge core tablets, CT-ES5 showed faster drug release, i.e., in 30 hours, including lag time. Hence, this was selected for carrying out the pharmacokinetic evaluation. Drug-excipient compatibility studies indicated no interaction of drugs with eudragits used in the study. The pharmacodynamic studies on the compression-coated tablet of mesalamine suitable for rat doses clearly indicated the superiority of compression-coated mesalamine colon-targeted drug delivery system. Pharmacodynamic studies like histopathology and macroscopic study indicated the suppression of disease to a great extent compared to the pure drug. Hence, it can conclude that local action is more predominant than systemic action, which is the more desirable parameter for colon targeting for treating the IBD using the compression-coated mesalamine microsponges.

Ethics Approval and Consent to Participate: The study was carried out per the approval of the Institutional Animal Ethical Committee (Reg. No. 516/01/A/CPCSEA) of A.U. College of Pharmaceutical Sciences, Andhra University, Vishakhapatnam, India.

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