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FORMULATION AND EVALUATION OF MICROSPONGES LOADED WITH ANTI-INFLAMMATORY DRUG SULFASALAZINE

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ABSTRACT

The present research is aimed to develop and characterize Sulfasalazine loaded microsponges-based novel colon-specific drug delivery systems in a view to bypass the upper gastrointestinal tract (GIT) for enhanced therapeutic effect. Microsponges were developed by quasi emulsion solvent diffusion method by using two different polymers Eudragit L-100 and Ethyl cellulose in the ratio of 1:1, 1:0.75, 1:0.5,1:0.25. Among these formulations two were selected, sieved and compressed into tablets. Then tablets were evaluated. The F4 was selected as optimizedformulation based on % entrapment efficiency of 94.56%, and % cumulative drug release of 94.76.Release studies revealed that microsponges prevented the premature release of Sulfasalazine in upperGIT andspecifically released the drug at colonic pH.

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INTRODUCTION

Inflammatory bowel disease (IBD) represents of two forms of intestinal disorders that cause prolonged inflammation of the digestive tract. It involves two major forms of chronic intestinal disorders namely Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease can cause inflammation in ileum and colon while Ulcerative colitis involves inflammation of the rectum and may affect a part or the entire colon. Colon drug delivery has gained importance for the delivery of the drugs for the treatment of Crohn's disease, ulcerative colitis, irritable bowel syndrome and constipation.

Microsponge Drug delivery system that can specifically control the release rate and target drugs to specific locations of the body have a major impact on the health care system[1].They are polymer-based microspheres, microscopic that can suspend or entrap a wide variety of substances, and can then be incorporated into a formulated product[2]. Microsponges are designed to deliver a pharmaceutical active ingredient efficiently at minimum dose, reduced side-effects, enhance stability and also to modify drug release profile. They are also capable of delivering pharmaceutical active ingredients efficiently at a minimal dose to targeted site, which reduces severe systemic degradation[3]. In oral applications microsponge system has been shown that rate of solubilisation of poorly water soluble drugs will be increased by entrapping such drugs in their pores [4]

Sulfasalazine(5-aminosalicylic acid) is an anti-inflammatory drug used to treat IBD[5], which is readily metabolized in the intestinal mucosal wall and in the liver and this metabolism can be overcome by formulating Sulfasalazine as colontargeted microsponges prepared using acid resistant polymers[6].

MATERIALS AND METHODS:

Sulfasalazine was obtained as a gift sample from Yarrow chem. products, Mumbai, India, while Eudragit L-100 was obtained as a gift sample from Kemphasol, Mumbai, India. Ethyl cellulose and polyvinyl alcohol were procured from S.D.Fine-Chem Ltd., Mumbai, India. Other chemicals and reagents used were of analytical grade and excipients used were of standard pharmaceutical grade.

Fourier-transform infrared (FTIR) spectroscopy[7]:

The Compatibility of drug and polymer was characterized by means of FTIR spectroscopy. The compatibility ofSulfasalzine,Eudragit L-100, Ethyl cellulose, and optimized formula was checked by using Bruker1.2.4 IR system (software).

Determination of λ_{max} and Calibration Curve for Sulfasalazine:

From the standard stock solution aliquots 2ml, 4ml, 6ml,8ml and 10ml were pipetted out into 100ml volumetric flask .The volume was made up with phosphate buffer pH 7.4 to get final concentration of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml, 100µg/ml respectively.

One of the above solutions i.e.,60µg/ml was selected for the determination ofλ_{max}. This solutionwas scanned between the range of 200-400nm.from the scan it was concluded thatthe $\lambda_{max}$of Sulfasalazine was 359nm.

The absorbance of each concentration was measured at λ_{max} 359nm using UV Visible spectrophotometer. Absorbance was measured at 359 nm against phosphate buffer of pH 7.4 as blank spectrophotometrically[8].

Drug-loaded microsponge preparation:

Microsponges were prepared by quasi-emulsion solvent diffusion method. It consisted of two phases, the internal and the external phase. Polymer(Eudragit L-100 or Ethyl cellulose) and plasticizer(Glycerine) was dissolved in a organic solvent(Ethanol) to form the internal phase. Sulfasalazine(drug) was added to the internal phase with gradual stirring (1000 rpm). The internal phase was then poured into the external phase containing Polyvinyl alcohol solution in water. After 8 h of stirring, the formed Sulfasalazine loaded microsponges were filtered and dried at 40° for 12 h. Various formulation batches were prepared as shown in Table 1[9].

Table 1: Compostion of sulfasalazine loaded Microsponges.

FORMULATION / INGREDIENTS(Mg)	F1(1:1)	F2(1:0.75)	F3 (1:0.5)	F4 (1:0.25)	F5 (1:1)	F6 (1:0.75)	F7 (1:0.5)	F8 (1:0.25)
Drug(mg)	400	400	400	400	400	400	400	400
Eudragit(mg)	400	300	200	100	-	-	-	-
Ethyl cellulose(mg)	-	-	-	-	400	300	200	100
Glycerine (ml)	1	1	1	1	1	1	1	1
Ethyl alcohol (ml)	20	20	20	20	20	20	20	20
PVA (mg/ml)	50	50	50	50	50	50	50	50
Distilled water(ml)	50	50	50	50	50	50	50	50

Evaluation of Sulfasalazine loaded microsponges:

The morphology and surface characteristics of the microsponges was studied using scanning electron microscopy (SEM) by coating Sulfasalazine loaded microsponges with gold palladium alloy under vacuum. Coated samples were then examined using LEO 430 SEM analyser [10-12].

Determination of Drug Entrapment efficiency [13]:

For the determination of encapsulation efficiency (EE), 100mg of Sulfasalazine loaded microsponges was placed in 100 ml of 7.4 pH phosphate buffer for 12 h with continuous stirring. The samples were filtered and analysed at 390 nm against blank on a UV spectrophotometer. The EE was calculated using the following Equation:

Entrapment Efficiency = mass of drug in microsponge/initial mass of drug×100

***In- vitro* Drug release study:**

In- vitrodrug release studies was carried out in a USP paddle apparatus with stirring rate 100 rpm at 37±0.5°. Drug release was carried out in 900 ml of pH 7.4 phosphate buffer. Samples were withdrawn at regular intervals of time and each time were compensated by adding equal volume of fresh dissolution medium to maintain the sink condition. The samples was analyzed spectrophotometrically at a wavelength of 359 nm.

Formulation of Sulfasalazine loaded Microsponge tablets procedure[14]:

The Optimized Sulfasalazine loaded microsponges were further compressed into core tablets containing 300 mg drug and other excipients like lactose and magnesium stearate using the direct compression technique. All tablet constituents was weighed and mixed in a mortar for 15 min. The final powder mix was compressed using round flat punches on a tablet punching machine by applying required compression pressure.

Table:2 Composition of microsponge compressed core tablets:

Tablet formulation code	Microsponge formulations (mg)		Lactose (mg)	Magnesium stearate (mg)
F4	350	-	142	8
F8	-	390	102	8

Coating of colon-specific tablets[15]:

The coating solution was prepared by dissolving Eudragit S 100 in isopropyl alcohol:acetone mixture to this mixture TEC was added and mixed uniformly. Sulfasalazine loaded microsponge compressed tablets were coated with this ES solution by Dip coating method.

Table:3Composition of Dip coating solution.

S.NO	Ingredients	Weight
1.	Eudragit S 100	2.98gm
2.	Isopropyl alcohol: acetone	20:14.5ml (v/v)
3.	Tri-ethyl citrate	0.5ml

Evaluation parameters of microsponge-loaded tablets:

Pre-compression Studies:

Angle of repose:

Angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizantal plane. The flow characteristics was measured by using angle of repose. The funnel was fixed at height of 2 cm from a horizontal plate. After the adjustment is done the thumb is removed and the powder is allowed to flow over the plate to forma pile. The height of the pile was noted. A circumference was drawn with a pencil on a graph paper and the radius of base of a pile was measured at 5 different points.

Carr's consolidation index :

This property is also known as compressibility. It is indirectly related to the relative flow rate, cohesiveness and particle size. It is simple, fast and popular method of predicting powder flow characteristics. It was calculated by using following formula.

$$\text{Consolidation index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

Hausner's ratio:

A similar index has been defined by hausner in order to determine the flow property .If greater than 1.25 hausner's ratio is considered to be an indication of poor flow ability. It was calculated by using following formula.

$$\text{Hausners ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Post-compression Parameters:

Thickness:

Three tablets were picked from each formulation randomly and thickness was measured individually.It is expressed in millimeter and standard deviation(SD) was also calculated.

Hardness:

The hardness of the tablets was determined using a Pfizer hardness tester. It is expressed in kg/cm2. Three tablets were randomly picked and hardness of the same tablets from each formulation was determined. The mean and SD values were also calculated. Monsanto hardness tester was used to evaluate the hardness of tablet.

Friability:

Friability of tablets was determined by using Roche Friabilator. Ten tablets were randomly selected, weighed ($W_{initial}$) and transferred into the friabilator. The friabilator was operated at 25 rpm for 4 min or 100 revolutions. Tablets were weighed again (W_{final}). The % friability was calculated using thefollowing equation

$$\mathbf{F = W_{initial} - W_{final} / W_{initial} \times 100.}$$

Weight variation test:

For the weight variation test, 20 tablets were selected randomly from each formulation and weighed individually to check for weight variation. Weight variation was calculated using the following equation.

% deviation = average weight–weight of tablet/average weight×100.

Drug content:

100 mg of Sulfasalazine loaded microsponges was taken and mixed with 100 mL of 7.4 pH phosphate buffer. Stirring was carried out for four hours then the mixture was filtered, then filtrate was taken for absorbance.

***In-Vitro* release Studies of tablets:**

The coated tablets were prepared by dipping core tablets in ES coating solution. The coated tablets were also evaluated similarly for weight variation, thickness, hardness, and friability. *In- vitro* release studies were carried out in a USP basket apparatus with stirring rate 50 rpm at 37±0.5°. Initial drug release was carried out in 900 ml of 0.1 N HCl for 2hrs followed by in phosphate buffer pH 6.8 for 4hrs and in pH 7.4 buffer. Samples were withdrawn at regular intervals of time and each time were compensated by adding equal volume of fresh dissolution medium to maintain the sink condition. The samples were analyzed spectrophotometrically at a wavelength of 359 nm.

In-Vivo Study:

Acetic Acid Induced Experimental Ulcerative Colitis in Colon:

Wistar Albino rats (body weight = 160–200 g),*n* = 5, were selected and were caged individually with food and water *ad libitum*. As per prior approval of Institutional Animal Ethical Committee animal studies was conducted. .The rats were distributed randomly in three groups, that is, control, Sulfasalazine, and microsponges of Sulfasalazine, each comprising five animals. 1mL of acetic acid was given to induce ulcerative colitis in rats through intrarectal route which resembled with the inflammatory bowel disease in all groups . For three days, they are caged without any treatment to maintain the development of full IBD model. Each group received 1% carboxymethyl cellulose (w/v) solution orally. Group 1 received vehicle only, group 2 received Sulfasalazine (20mg/kg), and group 3 received Sulfasalazine microsponges. Animals were sacrificed after 24 h. The colon portion of mice was isolated and washed with saline, photographed. Further the histopathological study was performed on sections of colon tissues. Tissue samples were excised from each colon and maintained in 10% (v/v) formalin in saline for histopathological evaluation. The preserved colonic sections were fixed on paraffin slide and analyzed by microscope.

RESULTS AND DISCUSSION:

The FT-IR spectra of pure drug Sulfasalazine, pure polymer Eudragit L-100,Ethyl cellulose and other excipients are shown in Fig.1,2,3,4

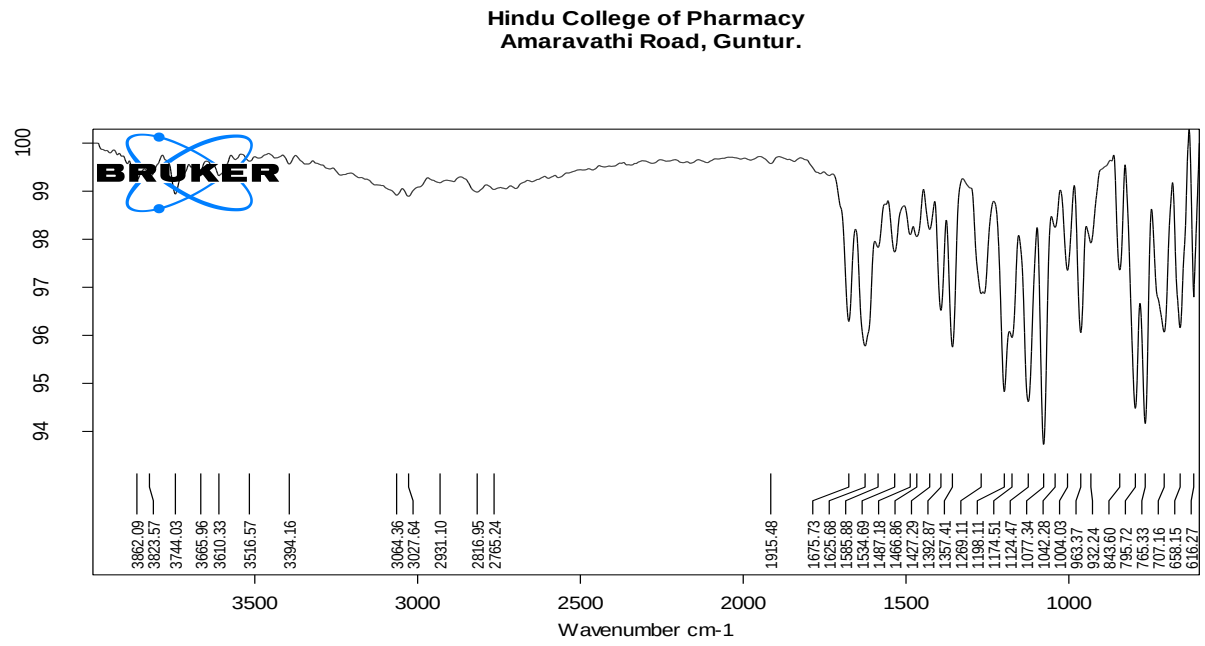


Fig.1FT-IR spectrum of pure drug Sulfasalazine.

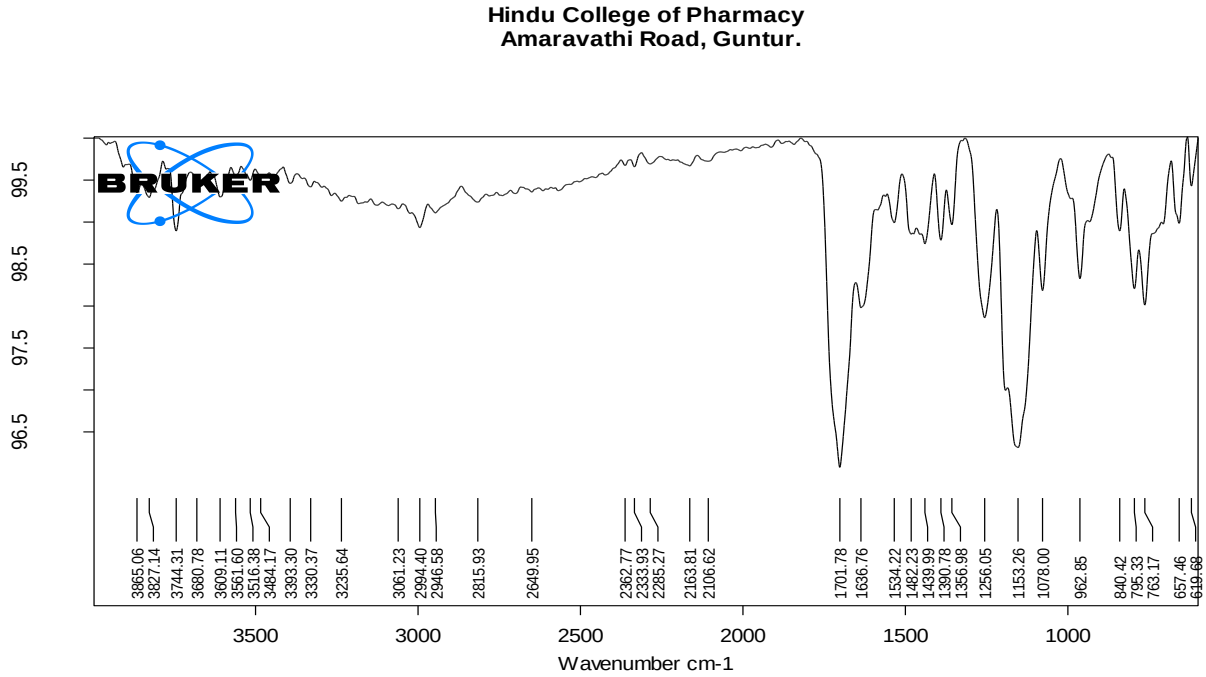


Fig.2 FT-IR spectrum of Drug + Eudragit L-100.

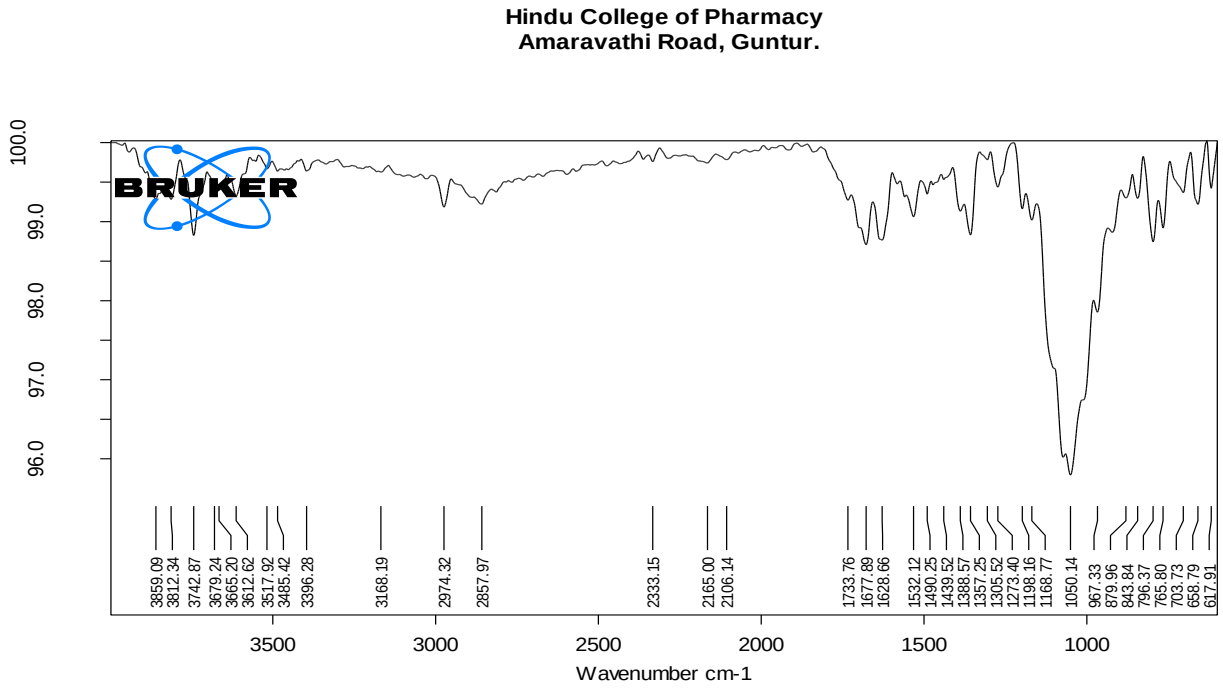


Fig.3FT-IR spectrum of Drug + Ethyl cellulose.

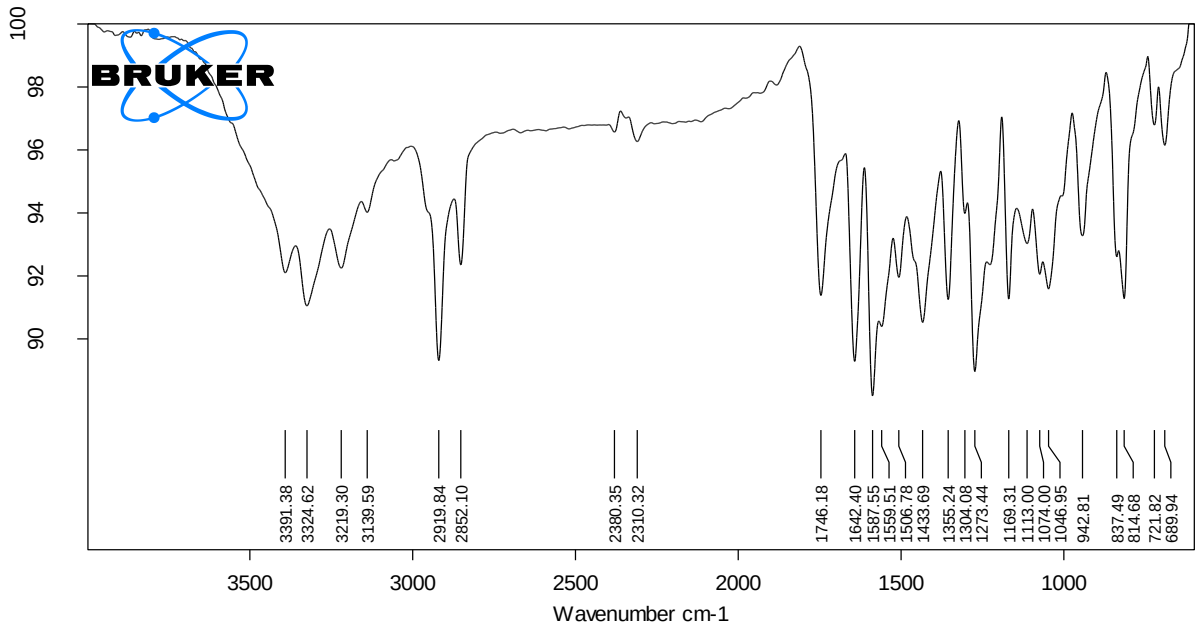


Fig.4 FT-IR spectrum of Drug + other excipients.

The presence of peaks at 1357.41cm^{-1}(Sulfones, S=O), 3394cm^{-1}(Amines and Amides, N-H)and 1675.73 cm^{-1} (Imines,Oximes, C=N), 1174.51 cm^{-1}(Amines, C-N) were characteristic to the pure Sulfasalazine. IR spectrum of Microsponge formulation showed peaks at1355, 3391.38, 1642.40 and 1113 cm^{-1}. IR spectrum reveals that there was no appreciable change in position and intensity of peak with respect to IR spectrum of pure Sulfasalazine. IR analysis revealed that there was no known chemical interaction between drug and excipients.

Standard calibration curve of Sulfasalazine:

Standard plot of Sulfasalazine was plotted as per the procedure in experimental methods and its linearity was shown in figure.6 . The standard graph of Sulfasalazine shows good linearity with R^2 value of 0.998, which indicates that it obeys beer-lamberts law in the concentration range 2-10µg/ml.

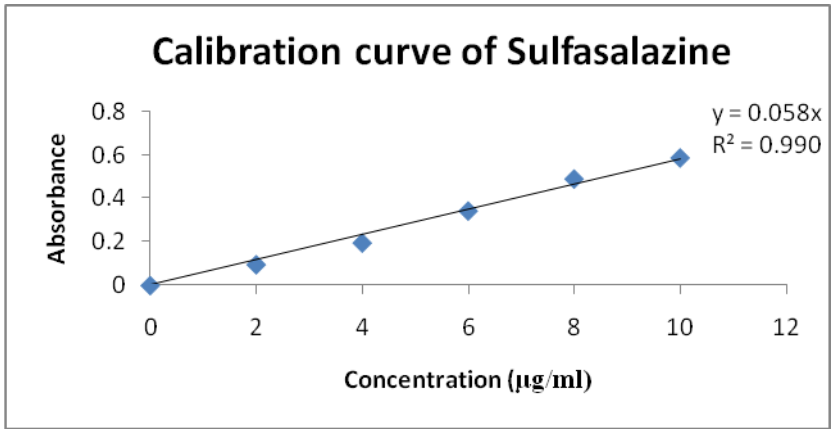


Fig.5Calibration curve of Sulfasalazine.

SEM Studies of Optimised formulation:

SEM images of Sulfasalazine revealed that the microsponges were spherical in shape and discrete with sharp boundaries having large internal aqueous space. SEM images of Microsponges produced from optimized formulation (F4).

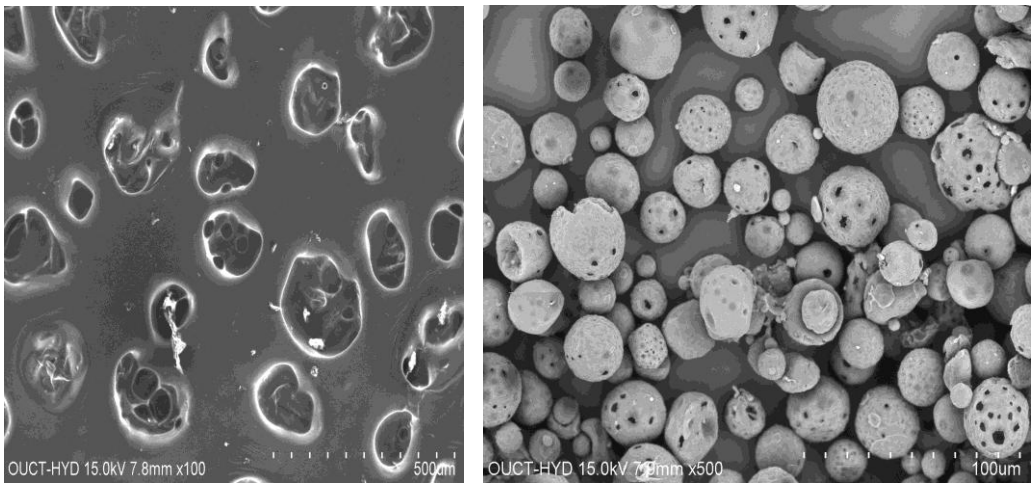


Fig.6 SEM images of Sulfasalazine loaded microsponges F4.

Drug encapsulation efficiency:

Encapsulation efficiency of Microsponge formulations ranges from 81.41% to 94.56 %. The drug encapsulation efficiency of all eight formulations of microsponges was shown in (table 4). Microsponges prepared by Eudragit L-100 having greater encapsulation efficiency compared to Microsponges prepared by Ethyl cellulose. As the Particle size decreases, in the formulation encapsulation of drug also increased. The (F4) formulation containing 1:1 ratio of Drug: polymer prepared by Quasi emulsion solvent diffusion method shows high encapsulation efficiency.

Table:4 Characterization of Microsponge formulations for Encapsulation efficiency.

Formulation Code	Encapsulation Efficiency (%)
F1	81.41±0.23
F2	84.53±0.54
F3	86.56±0.18
F4	94.56±0.95
F5	80.9±0.25
F6	82.58±0.34
F7	89.73±0.12
F8	90.54±0.52

Drug dissolution Profile of Microsponge formulation:

On comparing the dissolution profiles ofEightformulations, one was selected from each batch i.e. F4 from batch one, F8 from batch two. On comparing the two selected formulations, it was found that F4 released 94.76%, F8 released 91.23% of the drug at the end of 24 h period. The optimized microsponges were directly compressed in to tablets and then hardness, weight variation, thickness, friability and *in vitro* dissolution of these tablets were evaluated.

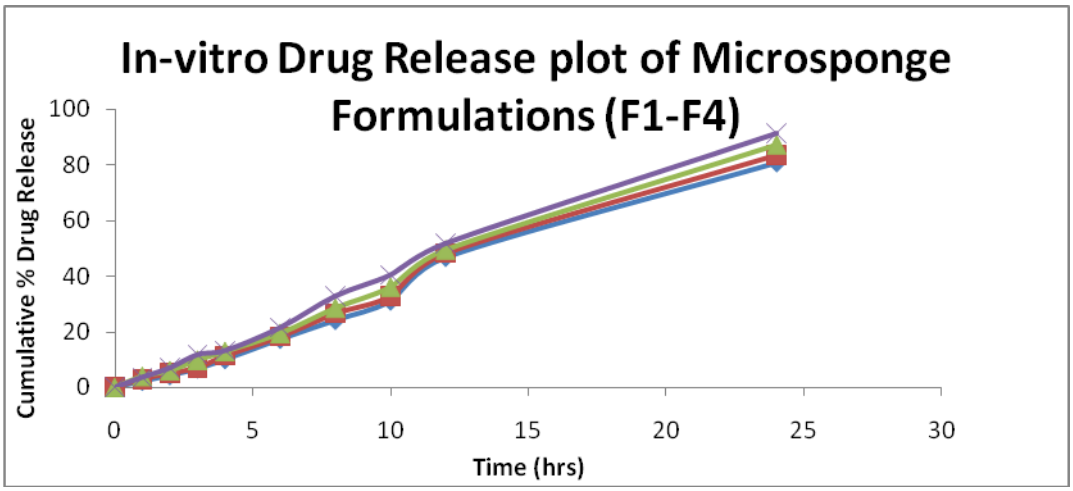


Fig.8 In-vitro drug release profiles of Microsponge formulations (F1-F4).

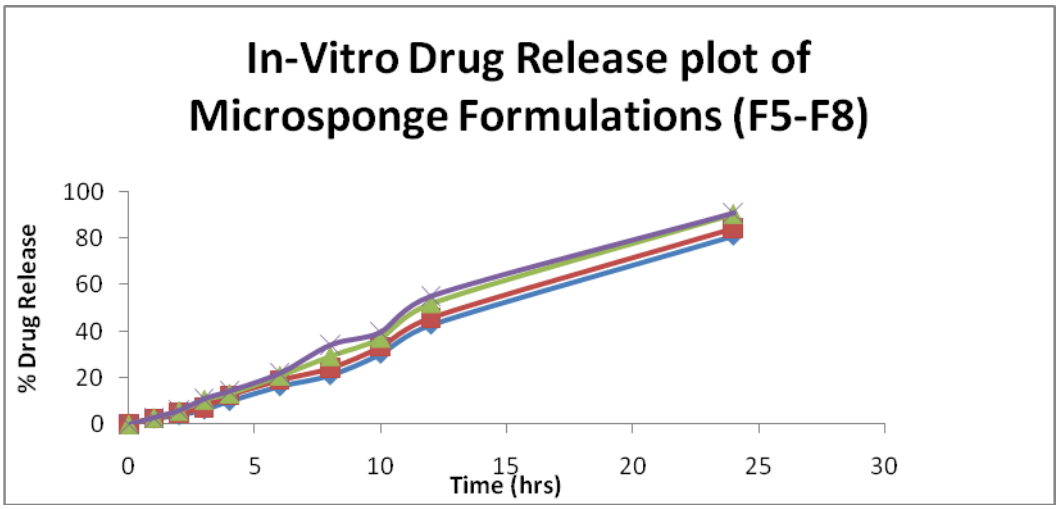


Fig.9 In-vitro Drug release profile of Microsponge formulations (F5-F8).

Characterization of Sulfasalazine loaded Microsponge tablets:

Sulfasalazine loaded Microsponge tablets was conducted for pre-compression studies as well as post-compression studies, *In-vitro*,*In-vivo* studies.

Table: 5 Pre-compression Studies.

Formulation code	Angle of repose	Carr's Index	Hausner's ratio	Type of flow
F4	20.14±0.33	13.46±0.23	1.19±0.28	Good
F8	23.87±0.68	15.321±0.51	1.21±0.34	Good

Table:6 **Post –compression studies.**

S.NO	BEFORE COATING		AFTER COATING	
	F4	F8	F4	F8
Thickness (mm)	3.6	3.4	3.7	3.5
Weight variation (mg)	490	483	510	507
Hardness (kg/cm^2)	4.85	4.31	5.2	5.1
Friability (%)	0.45	0.33	0.15	0.12
Drug content (%)	99.2	98.3	99.5	98.8

In-Vitro **Studies of tablets:**

The results of dissolution studies indicated that drug was not released in the first 5 h. After the lag time of 5 h, the drug started releasing at sixth hour in the presence of the colonic fluid. In the sixth hour drug release from different microsponge formulations i.e, F4 released 95.63% of drug at the 24th h, F8 released 92.30% of drug in 24 h. Figures showed the drug release profiles of tablets prepared. From the data obtained, it could be concluded that the formulation F4, which released 95.63 % drug in 24 h was the best formulation.

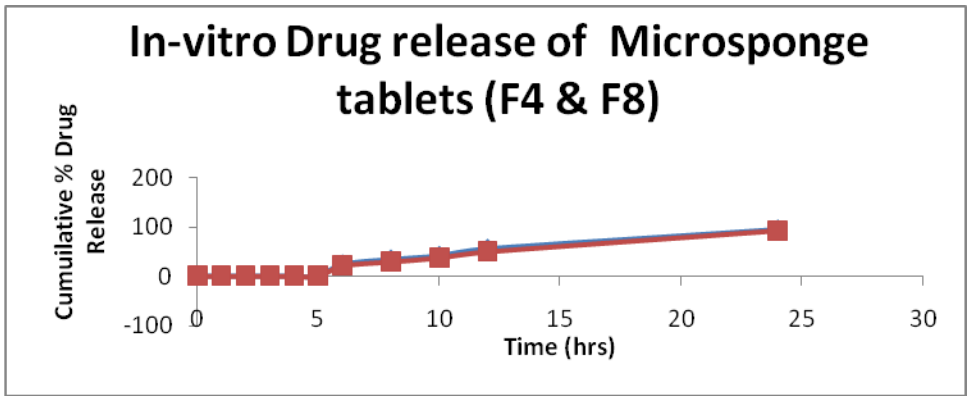
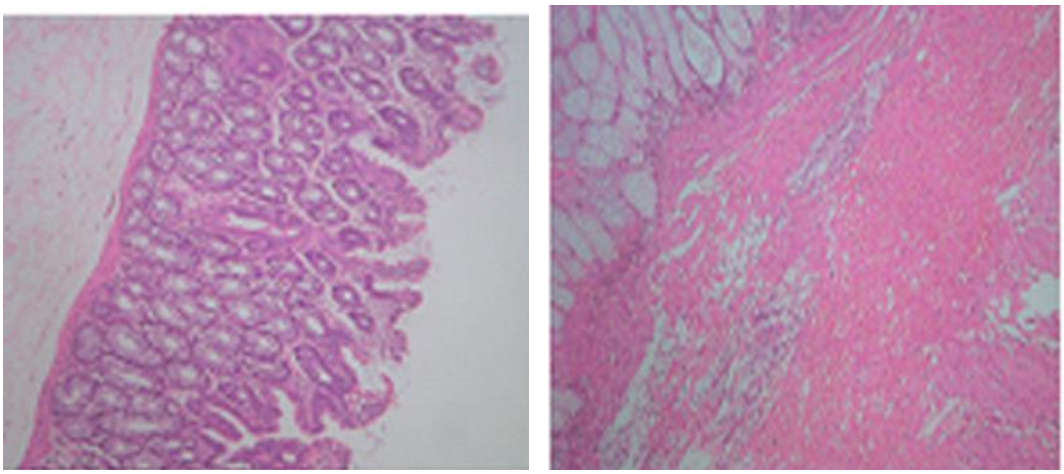


Fig.10 Cumulative percent Drug release of Microsponge tablets (F4 & F8).

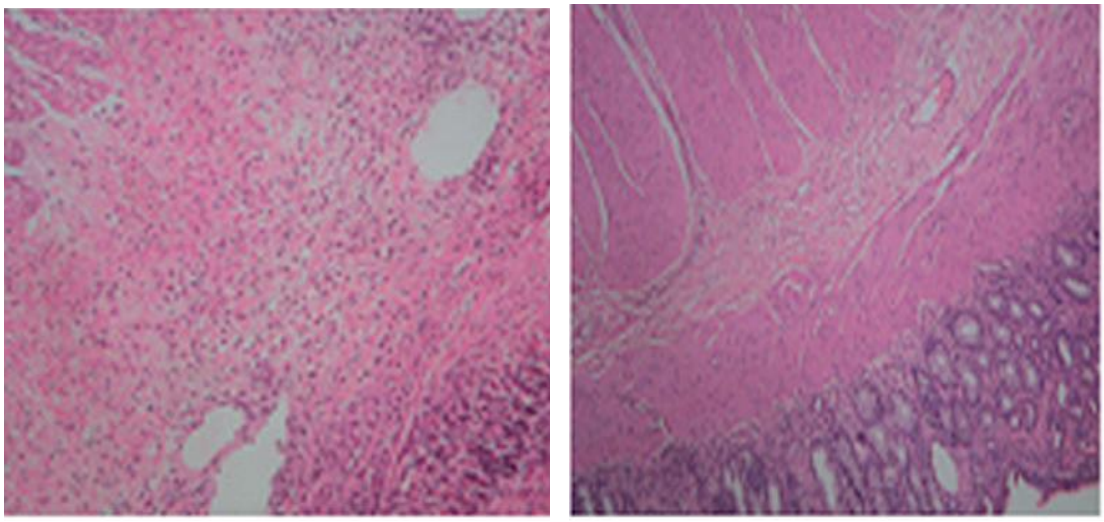
In-vitro drug release and release mechanism were studied for these two formulations. Release kinetics followed zero order kinetics and the release mechanism was observed to be the diffusion mechanism. The type of diffusion was super case-II transport, hence the prepared microsponge tablets F4 & F8 were found to release the drug as expected. When these two formulations were compared with each other, F4 was found to release the drug to the maximum extent (95.63 %) at the end of 24 h.

In-Vivo Study:

In vivo study using acetic acid induced colitis model in rat showed that ulcers in Sulfasalazine loaded microsponges treated group were recovered up to a good extent as compared to control group while in pure Sulfasalazine treated group the healing was mild. Results are shown as follows.



a) Normal mice colon **b)Acetic acid induced Colitis**



c) Treatment with marketed product **d) Treatment with Microsponge loaded with anti-inflammatory drug sulfasalazine.**

Fig.11 Histology of colonic sections.

CONCLUSION

The result of the present research work demonstrated that colon-targeted Sulfasalazine-loaded microsponges to serve the purpose of increasing the drug release by using ethanol and Eudragit/ Ethyl cellulose as the internal phase and PVA as the external phase. Eight microsponges were prepared using two different polymers Eudragit and Ethyl cellulose. Among these, best formulations was selected by their dissolution profiles, Entrapment efficiency. The formulations, F4 and F8 gave acceptable results with dissolution rate of 94.74% & 91.28% and entrapment efficiency of 94.56% and 90.54%. After compression into tablets and coating with Eudragit S-100, F4 gave best release rate of Sulfasalazine, with highest percent of the drug released at the end of 24 h i.e, 95.68% compared to the other tablet formulation. It was found that the drug release followed zero order release kinetics and diffusion with case-II transport mechanism. The *in-vivo* studies also revealed better therapeutic outcomes as compared to pure Sulfasalazine. These results indicated that microsponges could be used as efficient means of formulation to enhance drug delivery and bioavailability of a drug in the colon and this approach could produce efficient carriers for colon targeting.

Conflicts of interest:

There are no conflicts of interest.

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