Payal Deshmukh *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.7 Issue. 10, October- 2022, pg. 119-135

ISSN: 2519-9889
Impact Factor: 5.721

Probiotic-Assisted Colon-Specific Delivery of Anti-Inflammatory Drug - 5 ASA

Payal Deshmukh; Nikita Upadhyaya; Sunita Patidar; Rajat Pawar
Swami Vivekanand College of Pharmacy, Indore
DOI: 10.47760/ijpsm.2022.v07i10.007

Abstract:

Background: Mesalamine, one derivative of 5-aminosalicylic acid (5-ASA), has been recommended as the first-line medicine to induce and maintain remission in patients with mild-to-moderately active ulcerative colitis on account of its efficacy and safety.

Objective: To evaluate the efficacy and safety of mesalamine in conjunction with probiotics for ulcerative colitis an ideal colon targeting system aims to deliver a therapeutic agent, selectively and effectively, to the colon. This system should ideally retain the drug release in the upper GI tract (stomach and small intestine); while trigger the drug release in the colon. Several approaches have been used to fabricate formulations to achieve a colon specific delivery of mesalamine such as; time dependent, enzymatic/microbial responsive approaches. This overview outlines the recent advances in mesalamine-colon delivery approaches for the potential treatment of ulcerative colitis and Crohn' disease.

Method: The matrix tablets of guar gum were prepared by wet granulation method Lactose, guar gum, talc and magnesium stearate were sifted separately through sieve number 60 to obtain particles of uniform size. Weighed quantity of Mesalamine was sifted through sieve number 100.

Result: Matrix tablets containing Mesalamine were prepared by wet granulation method using guar gum as the colon targeting polymer and varying concentrations of probiotics Sporlac and Vivaflora to assist in colon targeting

Keywords: Colon, 5 ASA, guar gum, matrix tablet, probiotics.

IJPSM

Payal Deshmukh *et al*, International Journal of Pharmaceutical Sciences & Medicine (IJPSM), Vol.7 Issue. 10, October- 2022, pg. 119-135

ISSN: 2519-9889
Impact Factor: 5.721

1. Introduction:

Probiotics are live bacteria and yeast that are good for health especially our digestive system, probiotics are often called good or helpful bacteria .5 amino salicylic acid is an active moiety in ulcerative colitis , but it is not effective orally because of inability to reach the large bowel (it is absorbed in the small intestine) it has been formulated as delayed release preparation by coating with acrylic polymer.5-ASA is the main anti-inflammatory compound that's act locally in colon. It is first line of treatment in mild to moderate ulcerative colitis &crohn's disease. When given alone 5-ASA is absorbed > 80% in proximal intestine and very little reaches up to colon.The purpose of this formulation to reduce dosing frequency and delayed delivery to a time appropriate to treat active phase of disease and to ability to cut down the conventional dose[1]

Mesalamine, lactose and guar gum was mixed together and blended with addition of water (q.s) for granulation. The wet mass was passes through sieve number 14 and the granules were allowed to dry at 50°C in a tray drier for 2 h. The dried granules were passed through sieve number 16 to obtain a mixture of granules and fines. Magnesium stearate and talc were added to the granules and blended in a double cone blender for 5 min. The lubricated granules were compressed using Cadmach tablet punching machine. In table presents the composition of Mesalamine tablets Oral drug delivery system is the most commonly used route for drug delivery due to its ease of administration, better patient compliance, and flexibility in design and development of formulation. The drug delivery to the colon has attracted a lot of attention of the scientist working on oral drug delivery system which is mainly due to the fact that colon is a site where both local and systemic drug delivery can take place . In recent times, thecolon-specific drug delivery systems are also gaining importance for the systemic delivery of proteins and peptide drugs[2]. Due to negligible activity of brush border membrane peptidase activity and less activity of pancreatic enzymes, the colon is considered to be more suitable for delivery of protein and peptide in comparison to small intestine The aim of present work was Multiple 5-aminosalicylic acid (5-ASA) is the active moiety of sulfasalazine in the treatment of chronic inflammatory bowel disease, several new 5-ASA based drugs have been developed. These consist either of slow- or delayed-release formulations of plain 5-ASA or sulpha-free azo compounds of 5-ASA. The different formulations and compounds have varying bioavailability, which makes it possible to use them alternatively in different clinical situations. A review of the literature is given, together with suggestions as to how the new drugs might be used in different clinical situations[3].

ISSN: 2519-9889
Impact Factor: 5.721

2. Material and Method:

Mesalamine was obtained as generous gift sample from WellonaPharma Gujarat. Talc, magnesium stearate, lactose monohydrate, and guar gum were purchased from Oxford Fine Chemicals LLP Mumbai. Sporlac sachets and Vivaflora capsules were purchased from local Pharmacy store of Bhopal. All other chemicals and reagents used were of analytical grade and used as obtained.

2.1 Organoleptic Properties

Characterization of Mesalamine[4]

The physical characterization of the drug was performed according to the reported procedure andthe results obtained were compared with that of the standard specifications (Table .1).

Table .1: Organoleptic Evaluation of Mesalamine

S. No	Parameter	Observation
1	Physical appearance	Greyish-White powder
2	Odour	Odourless

Result: The physical Appearance were Greyish-White powder, odor of the obtained drug sample were observed odourless with the help of the sensory organs.

Determination of λmax: Accurately weighed 5 mg of Mesalamine was dissolved in 5 mL of PBS pH 7.4 in a 100 mL volumetric flask. 1 mL of this stock solution was pipetted out in to a 10 mL volumetric flask and volume made up to the mark with PBS pH 7.4. The resulting solution was then scanned between 200-400 nm using UV spectrophotometer. The λ_{max} was found to be 337 nm. After 3 days of storage at room temperature the solution was again scanned and it was found to be unchanged ethanol and acetone[5].

ISSN: 2519-9889
Impact Factor: 5.721

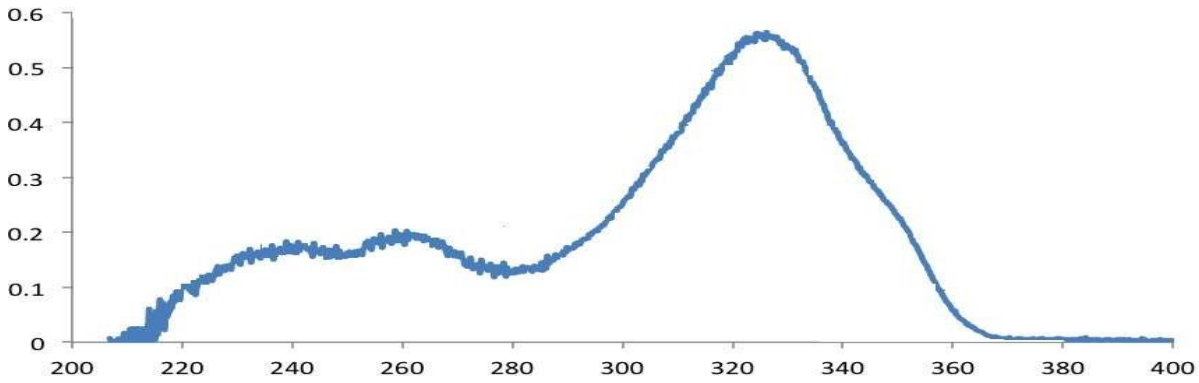

Figure 1 UV spectra of Mesalamine

Result: The absorption maximum of Mesalamine in PBS pH 7.4 was found to be 337 nm

FT-IR- SPECTRO SCOPY FT-IR spectrum of the sample of Mesalamine was obtained and examined for the presence of characteristic peaks and matched with that of the reference spectra in databases for confirmation of the identity of the drug.

Table: 2 Vibration frequencies (FTIR) of Mesalamine

S. No	Wave number (Standard)	Occurs due to	Wave number (Sample)
1	3430	O-H stretching	3480
2	1650	C=O stretching	1652
3	1610	C=C stretching	1606
4	1495	O-H bending	1552
5	1450	C-H stretching	1439
6	1390	Aromatic C=C stretching	-
7	1360	C-H bending	-
8	1325	C-N stretching	1307
9	1275	C-O, C-N stretching	1280
10	1260	C-N stretching (aromatic amine)	-

ISSN: 2519-9889
Impact Factor: 5.721

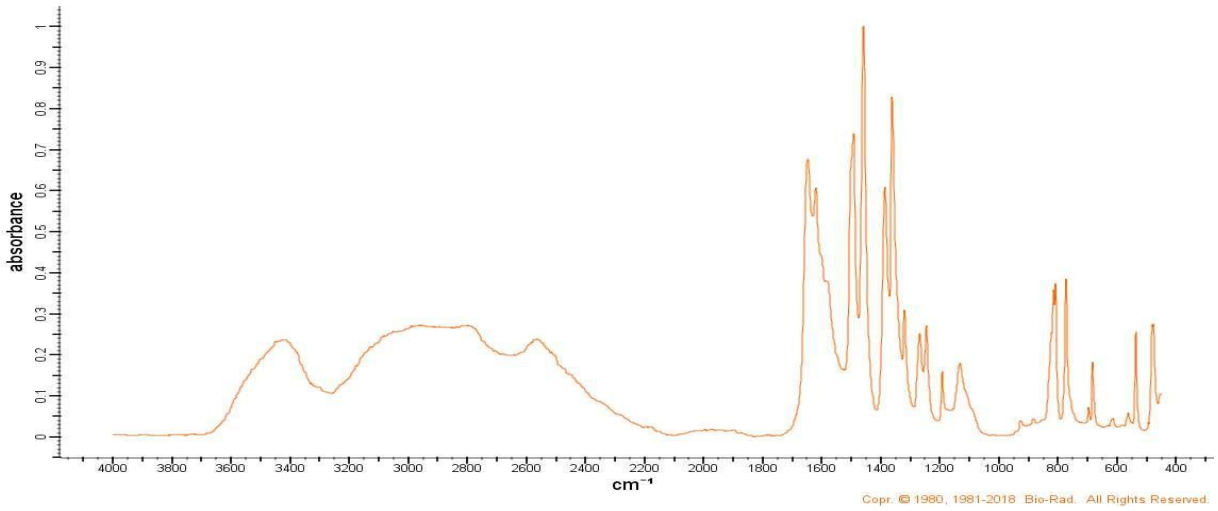

Figure 2 Standard FTIR spectra of Mesalamine (Available from Spectra base)

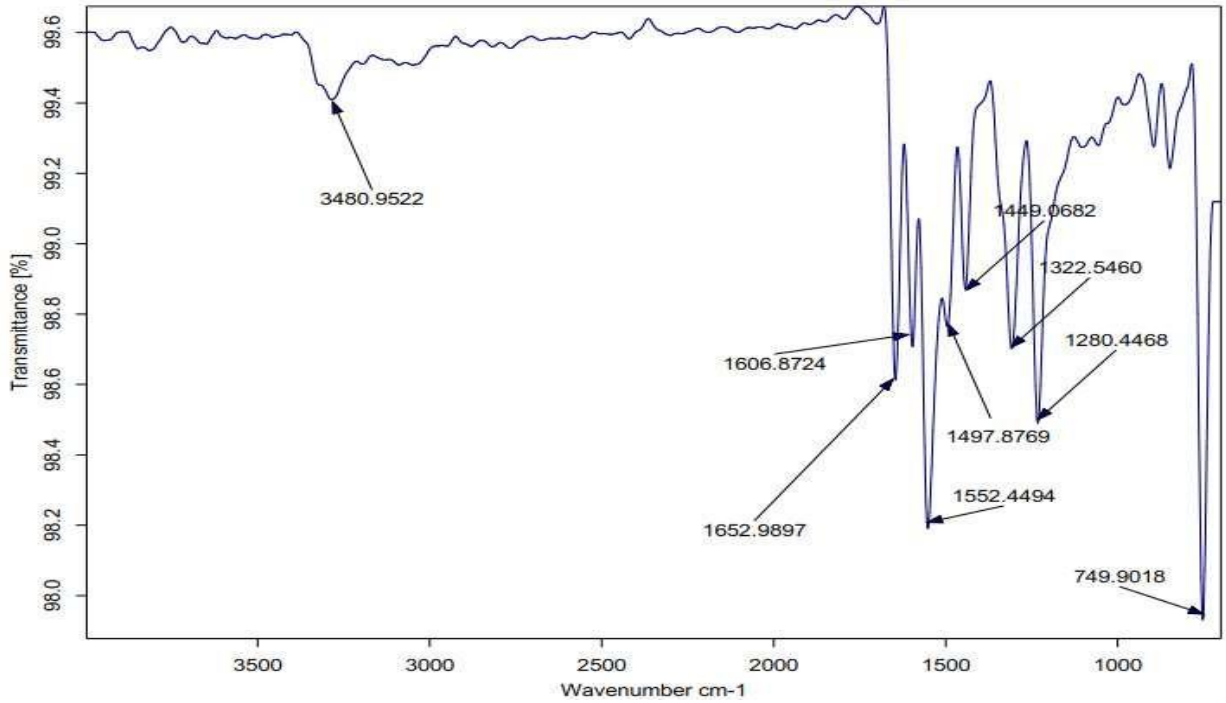

Figure 3 FTIR spectra of Mesalamine Sample (Pure Drug)

ISSN: 2519-9889
Impact Factor: 5.721

Result: The IR spectrum of the drug sample of Mesalamine was obtained on Bruker FTIR spectrometer and the peaks obtained were matched with the standard spectra available at spectrabase.com

Preformulation Studies:

Preformulation studies are an important tool for determination of physical and chemical properties of the drug before incorporating it in formulation development.

Solubility:

Approximately 1 g of drug was weighed accurately and transferred to different 10 ml volumetric flasks. Different solvents were added to the flask respectively and the flasks was observed for complete dissolution of the drug**.** Solubility was determined in different solvents like water, HCl,ethanol and acetone slightly soluble in Water more soluble in Hot Water[6].

Table: 3 Solubility profile of Mesalamine

S. No	Solvent	Solubility
1	Water	Slightly Soluble
2	Ethanol	Insoluble
3	HCl	Soluble
4	Acetone	Insoluble

Result: The drug Was Soluble in HCL and Slightly Soluble in Water, and insoluble in Ethanoland acetone.

Melting point determination: Melting point was determined by open capillary method and isuncorrected. A small quantity of powder was placed into fusion tube and placed in

ISSN: 2519-9889
Impact Factor: 5.721

the melting point apparatus (Tempo, Mumbai). The temperature of the apparatus was gradually increased and the temperature at which the powder started to melt and the temperature at which all the powder got melted was recorded[7].

Result: Melting point of mesalamine was found to be 283º C

Preparation of Calibration Curve in Phosphate Buffer Solution pH 7.4 : Accurately weighed 25 mg of Mesalamine was taken in 25 mL volumetric flask and dissolved in PBS pH 7.4 and volume was made up with PBS pH 7.4 to the mark .This resulted 1000 µg/mL stock solution. From the above stock solution 10 mL was taken in another 100 mL volumetric and volume was made up with PBS pH 7.4 to mark and the concentration of solution become 100 µg/mL. After that from the above solution the aliquots of 1-10 mL of stock solution were taken into a series of 10 mL volumetric flask and volume was made up to the mark with PBS pH 7.4 and it was analyzed at λ max 337 nm using UV spectrophotometer. The standard curve was plotted between absorbance and concentration[9].

Table 4: Absorbance of Mesalamine in PBS pH 7.4

S.No	Concentration (µg/mL)	Absorbance
1	10	0.069
2	20	0.131
3	30	0.189
4	40	0.247
5	50	0.310
6	60	0.380

ISSN: 2519-9889
Impact Factor: 5.721

7	70	0.458
8	80	0.521
9	90	0.596
10	100	0.665

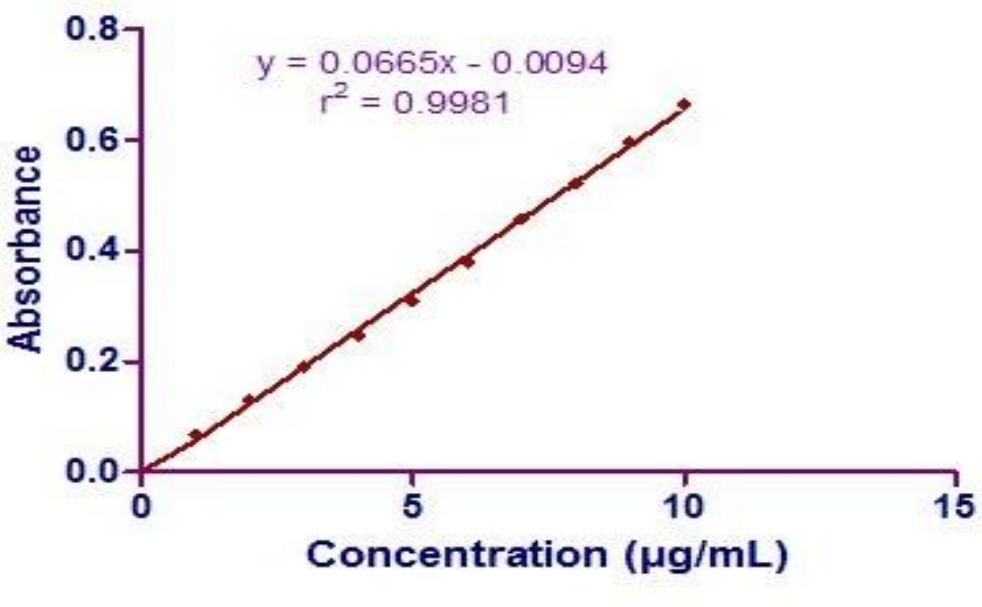

Figure: 4 Standard curve of Mesalamine

In vitro digestion of guar gum by probiotics: Slurry of guar gum (1% w/v) was prepared by dispersing 2 g of guar gum in 200 mL distilled water. To the slurry was added the contents of one sachet of Sporlac (1 gm) and one capsule of Vivaflora separately and the mixture were incubated at 37°C in incubator for a period of 24 h. At various time interval the change in pH and viscosity was measured for each dispersion using calibrated pH meter (Labtronics) and Brookfield viscometer, respectively. Guar gum dispersion (1% w/v) was used as the control sample for the study[10].

ISSN: 2519-9889
Impact Factor: 5.721

Table 5 Effect of probiotics on pH and viscosity of guar gum

Time (h)	Guar Gum		Guar Gum + Sporlac		Guar Gum + Vivaflora	
	pH	Viscosity (cps)	pH	Viscosity (cps)	pH	Viscosity (cps)
0	6.97	2560	6.98	2560	6.97	2560
1	6.97	2560	6.97	2560	6.98	2560
2	6.98	2560	6.97	2560	6.97	2560
4	6.98	2560	6.98	2560	6.98	2560
6	6.98	2560	6.98	2560	6.98	2560
8	6.97	2560	6.97	2560	6.98	2560
10	6.99	2560	6.81	2380	6.73	2400
14	6.98	2560	5.93	2200	5.96	2250
18	6.97	2560	5.77	1800	5.73	1830
24	6.97	2560	5.40	670	5.52	740

Result: The *in vitro* digestion study of guar gum in presence of probiotics was performed to assess the effect of intestinal microbial flora of guar gum. The pH and viscosity of guar gum 1% w/v solution was used as the marker for degradation of guar gum. The results obtained are presented in Table[11]

Partition coefficient: The partition coefficient of drug was examined in n-Octanol: Phosphate buffer pH 7.4 system. It was determined by taking 5 mg of drug in separating funnel containing 5ml of n-Octanol and 5 ml, PBS pH 7.4 buffer. The separating funnel was shaken for 2 h using a wrist action shaker and allowed to stand for 2h in order to equilbrate. The two phases separated and the amount of drug in aqueous phase was analysed spectrophotometrically at 337 nm after appropriate dilution with respective buffer.

ISSN: 2519-9889
Impact Factor: 5.721

The partition coefficient of drug was calculated using the following formula-

$$\text{Partition coefficient} = \frac{\textit{Amount of drug in octanol phase}}{\textit{Amount of drug in aqeuous phase}}$$

Result : The two phases separated and the amount of drug in aqueous phase was analyzed spectrophotometrically at 337 nm after appropriate dilution with respective buffer.

Formulation of matrix tablet of Mesalamine using guar gum[12]

The matrix tablets of guar gum were prepared by wet granulation method Lactose, guar gum, talc and magnesium stearate were sifted separately through sieve number 60 to obtain particles of uniform size. Weighed quantity of Mesalamine was sifted through sieve number 100.

Mesalamine, lactose and guar gum was mixed together and blended with addition of water (q.s) for granulation. The wet mass was passes through sieve number 14 and the granules were allowed to dry at 50°C in a tray drier for 2 h. The dried granules were passed through sieve number 16 to obtain a mixture of granules and fines. Magnesium stearate and talc were added to the granules and blended in a double cone blender for 5 min. The lubricated granules were compressed using Cadmach tablet punching machine. Table 5 presents the composition of Mesalamine tablets.

Formulation of matrix tablet of Mesalamine using guar gum-probiotic mixture

The matrix tablets of Mesalamine were prepared using guar gum (40% of tablet weight) using the method reported in section 4.4.3. The probiotics were added in two portions: half prior to granulation and the other half prior to final blending of the mixture. Table 6 presents the composition of Mesalamine tablets.

ISSN: 2519-9889
Impact Factor: 5.721

Table 6 Composition of matrix tablets

	Quantity of each ingredient per tablet in mg				
Ingredient	**MT1**	**MT2**	**MT3**	**MT4**	**MT5**
Meselamine	100	100	100	100	100
Guar Gum	100	200	200	200	200
Lactose	285	185	105	85	65
Magnesium Stearate	5	5	5	5	5
Talc	10	10	10	10	10
Sporlac	-	-	40	50	60
Vivaflora	-	-	40	50	60
Weigh of tablets	**500**	**500**	**500**	**500**	**500**

Evaluation of Pre Compression Parameter[13]

Angle of repose

Angle of repose was determined by using funnel method. Accurately weighed amount of microspheres were taken in funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the pile. The granules were allowed to flow through the funnel freely on to the surface. The diameter of powder cone was measured and the angle of repose was calculated using the following formula:

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

where h is the height of the pile; θ is the angle of repose; and r is the radius of the heap.

Bulk Density

The apparent bulk density (ρ_b) was determined by accurately weighing 10 g of the microspheres and transferring it to a 100 mL graduated cylinder. The volume occupied by the microspheres was determined and the bulk density was calculated using the formula:

ISSN: 2519-9889
Impact Factor: 5.721

$$\rho_b = M/V_b$$

where, $\rho_b$is the bulk density; M is the mass of the microspheres and V_b is the volume occupied by the microspheres.

Tapped Density

The measuring cylinder containing a known mass (M) of the microspheres was tapped for a fixed time and the volume occupied by the blend after tapping was measured. The tapped density $\rho_t$was calculated using the formula:

$$\rho_t = M/V_t$$

Hausner's Ratio

Hausner's ratio was calculated from the bulk and tapped density using the formula:

Hausner's Ratio = Tapped density/ Bulk density

Percent compressibility (Carr's Index)

The consolidation index (Carr's compressibility index) was determined by comparing the bulk density and the tapped density of the powder. Carr's compressibility index is calculated using the formula:

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} * 100$$

In Carr's Index, the value below 15% indicates good flow properties whereas a value above 25% indicates poor flow characteristics.

ISSN: 2519-9889
Impact Factor: 5.721

Table 7 Results of pre compression of various granular blends

Batch Code	Angle of repose	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Index
MT1	26°32'	0.43	0.51	1.18605	15.6863
MT2	26°97'	0.42	0.5	1.19048	16
MT3	27°48'	0.44	0.54	1.22727	18.5185
MT4	28°52'	0.46	0.55	1.19565	16.3636
MT5	27°37'	0.43	0.52	1.2093	17.3077

Post compression Parameter of matrix tablets[14]

Thickness

The thickness of 20 randomly selected tablets from each batch of formulation was measured using a digital verniercaliper.

Weight variation test

20 tablets were randomly taken and weighed to calculate the average weight of the tablets. Each of these tablets was individually weighed and the difference from average weight was calculated. The percent weight variation was calculated to determine the deviation from the average weight.

Friability test

The friability test of the formulations was performed using a Roche type friability test apparatus. Twenty tablets were initially weighed ($W_{initial}$) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or runup to 100 revolutions. The tablets were weighed again (W_{final}). The percentagefriability was then calculated by the formula

$$\% \text{ Friability} = \frac{W_{initial} - W_{final}}{W_{initial}} X\ 100$$

Hardness test

The hardness of the formulated tablets was tested using Monsanto type hardness tester. Three tablets from each batch of formulation were randomly taken and the force required to break the tablets was measured using hardness tester.

ISSN: 2519-9889
Impact Factor: 5.721

Drug content

Twenty tablets from each formulation were weighed to determine the average weight. These tablets were crushed in a mortar then the amount of powder equivalent to 25 mg of drug was transferred in 25 mL of PBS pH 7.4. 10ml from this stock solution was withdrawn and diluted up to 100 mLwith PBS pH 7.4. 2 mLof this stock solution was pipetted out and diluted to 10 mL to obtain concentration of 20 µg/mL. Absorbance of the resulting solution was measured at 337 nm using UV spectrophotometer and the concentration was determined using the calibration curve. The drug content was calculated by applying the dilution factor.

3. RESULTS AND DISCUSSION:

Matrix tablets containing Mesalamine were prepared by wet granulation method using guar gum as the colon targeting polymer and varying concentrations of probiotics Sporlac and Vivaflora to assist in colon targeting. The results obtained from various steps of the study are presented in the following sections.

***In vitro* digestion of guar gum by probiotics**[15]

The *in vitro* digestion study of guar gum in presence of probiotics was performed to assess the effect of intestinal microbial flora of guar gum. The pH and viscosity of guar gum 1% w/v solution was used as the marker for degradation of guar gum. The results obtained are presented in Table

Table 8 Effect of probiotics on pH and viscosity of guar gum

Time (h)	Guar Gum		Guar Gum + Sporlac		Guar Gum + Vivaflora	
	pH	Viscosity (cps)	pH	Viscosity (cps)	pH	Viscosity (cps)
0	6.97	2560	6.98	2560	6.97	2560
1	6.97	2560	6.97	2560	6.98	2560
2	6.98	2560	6.97	2560	6.97	2560
4	6.98	2560	6.98	2560	6.98	2560
6	6.98	2560	6.98	2560	6.98	2560
8	6.97	2560	6.97	2560	6.98	2560
10	6.99	2560	6.81	2380	6.73	2400
14	6.98	2560	5.93	2200	5.96	2250
18	6.97	2560	5.77	1800	5.73	1830
24	6.97	2560	5.40	670	5.52	740

ISSN: 2519-9889
Impact Factor: 5.721

In vitro drug release study

The *in vitro* release study was done for all the formulations to assess the time duration up to which the drug is released by the matrix tablets and to prove a sustained release from the matrix (Table). The % cumulative release was plotted against time to obtain the release kinetics equation for the formulations:

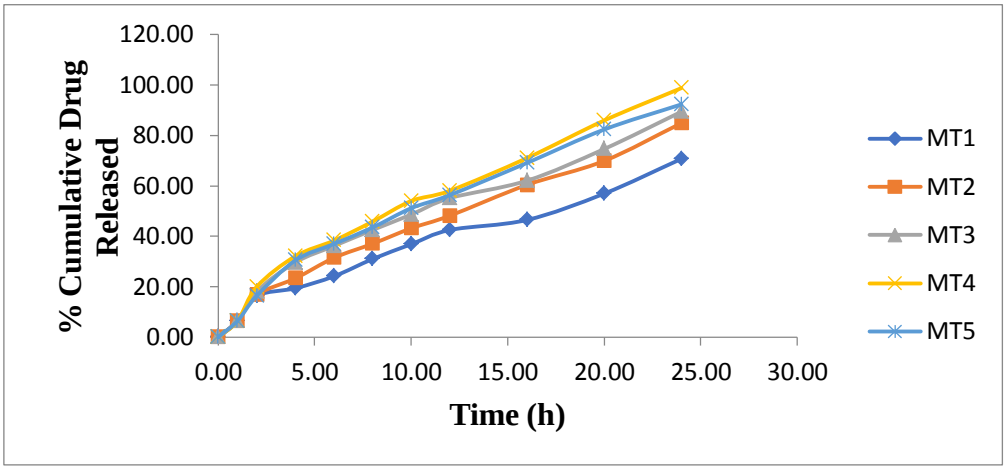

Figure 5 Cumulative percent of Mesalamine released from matrix tablets in absence of rat caecal content

Table 9 In vitro release profile of formulations in presence of rat caecal content

Time (h)	% cumulative release				
	MT1	MT2	MT3	MT4	MT5
0.00	0.00	0.00	0.00	0.000	0.000
1.00	6.43	6.43	6.43	6.433	6.433
2.00	18.18	21.90	18.91	19.044	17.116
4.00	23.30	29.75	31.81	37.731	32.610
6.00	28.09	32.74	38.73	46.708	42.386
8.00	34.34	39.66	46.18	54.422	53.292
10.00	39.73	45.44	58.74	61.804	72.776
12.00	51.50	56.82	69.45	78.961	99.443
16.00	67.92	65.86	85.08	99.842	99.908
20.00	78.03	82.22	91.33	99.842	-
24.00	84.95	95.98	98.64	-	-

ISSN: 2519-9889
Impact Factor: 5.721

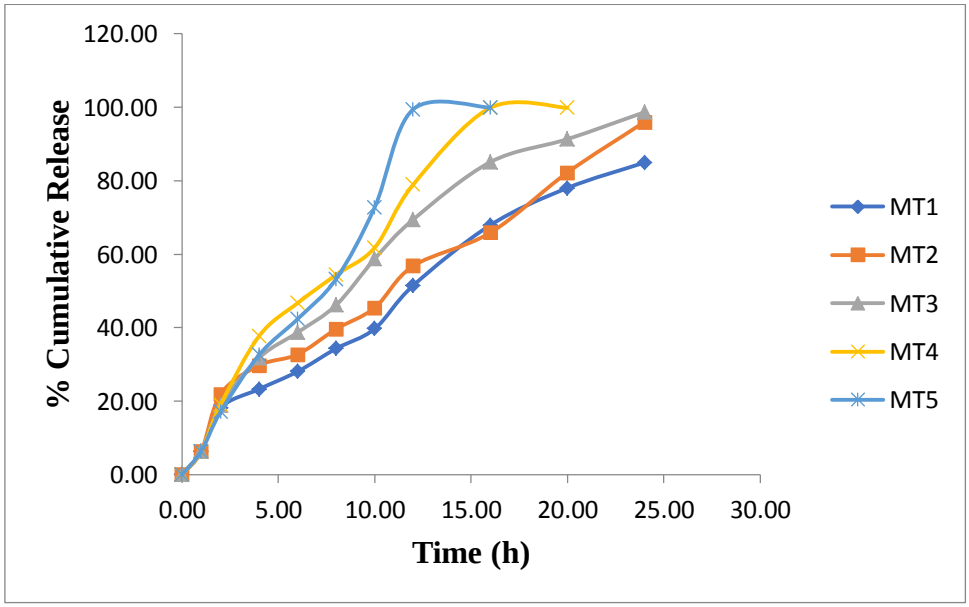

Figure 6 Cumulative percent of Mesalamine released from matrix tablets in presence of rat caecal content

4. CONCLUSIONS:

colonic release of the majority of drug even in the absence of colonic microflora and also In the present study, colon specific matrix tablets loaded with Meselamine were prepared using wet granulation method employing guar gum as the polymeric matrix and probiotics as the targeting aids. The results obtained showed that this methodology was able to produce produced sustained release of drug from the formulations.

Consequently, it can be concluded that the matrix tablets produced using the probiotic assisted procedure is an excellent delivery system that has good release behavior for actively releasing drug in the colon, and therefore, this system would provide a safe and effective strategy for treatment of ulcers of the stomach or other diseases of the gut.

5. ACKNOWLEDGEMENT:

Authors are thankful, for providing swami vivekanand college of pharmacy, Indore necessary financial support to carry out the present research work, Authors are also thankful to M/s.WellonaPharma Gujarat., for providing gift sample.

ISSN: 2519-9889
Impact Factor: 5.721

REFERENCES

[1] Abdullah, G. Z., Abdulkarim, M. F., Chitneni, M., Mutee, A. F., Ameer, O. Z., Salman, I. M., & Noor, A. M. (2011). Preparation and in vitro evaluation of mebeverineHCl colon-targeted drug delivery system. Pharmaceutical development and technology, 16(4), 331-342.

[2] Admed, I.S., & Ayres, J.W. (2011). Comparison of in vitro and in vivo performance of a colonic delivery system. International Journal of Pharmaceutics, 409 (1-2), 169-177

[3] Ahuja, V., & Tandon, R.K. (2010). Inflammatory bowel disease in the Asia–Pacific area: A comparison with developed countries and regional differences**.** Journal of DigestiveDiseases, 11, 134-147

[4] Amara AA, Shibl A. Role of Probiotics in health improvement, infection control and disease treatment and management. Saudi Pharmaceutical Journal 2015; 23: 107-114

[5] Asghar, L.F., & Chandran, S. (2006). Multiparticulate formulation approach to colon specific drug delivery: current perspectives. Journal of Pharmacy and Pharmaceutical Sciences, 9(3), 327-338

[6] Ashford, M. Fell, J., Attwood, D., Sharma, H., & Woodhead, P. An evaluation of pectin as a carrier for drug targeting to the colon. Journal of Controlled Release, 26 (3), 213-220

[7] Brahma, P.G., Thakur, N., Jain, S., Patel, P., Patel, D., Jain, N., & Jain, N.P. (2010). A Comprhensive Review On: Colon Specific Drug Delivery System (CSDDS). Journal of Pharmacy Research, 3(7), 1625-1629

[8] Challa, T., Vynala, V., &Allam, K. V. (2011). Colon specific drug delivery systems: A review on primary and novel approaches. International Journal of Pharmaceutical Sciences Review and Research, 7(2), 171-181.

[9] Chauhan P, Mehta N, Rathore MS, Jain A, Jain AK. Probiotic assisted colon targeted drug delivery system: research scope. Asian Journal of Pharmaceutical and Clinical Research. 2011, 4(2): 12-15

[10] Chien, Y.W. (1990). Controlled and modulated-release drug delivery systems. In: Swarbrick, J., Boyland, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, Inc., New York, pp. 281–313

[11] Chourasia, M.K., & Jain, S.K. (2003). Pharmaceutical approaches to colon targeted drug delivery systems. Journal of Pharmacy and Pharmaceutical Sciences, 6(1), 33-66

[12] Dodoo CC, Wang J, Basit AW, Stapleton P, Gaisford S. Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation. International Journal of Pharmaceutics 2017; http://dx.doi.org/10.1016/j.ijpharm.2017.07.068

[13] Ghosh PK, Gupta VB, Gondoliya B, Rathore MS. Probiotic-assisted colon-specific delivery of diclofenac sodium from guar gum matrix tablets: In vitro evaluation. Asian Journal of Pharmaceutics. 2010, 4(4): 173-178

[14] Goindi, S. Mann, K., & Aggarwal, N. (2011). Gastro-retentive floating beads of curcumin β-cyclodextrin complex to treat stomach tumors. Alternative Medicine Studies, 1 (e12), 49-53

[15] Gulati M, Singh S, Duggal S, Satyakam R, Sharma M. Improved oral targetted drug delivery system. International Patent Number. 2012, WO 2012/035561

