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Formulation Design and Characterization of Colon-targeted Mesalamine Microspheres and their Biodistribution Potential Study in Mice

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Abstract

Aim: The aim of this study is to design formulations of mesalamine microspheres (MMS) for the treatment of Crohn's disease and ulcerative colitis in the colon. **Materials and Methods:** Emulsification solvent diffusion method was employed for the preparation of MMS coated with Eudragit RS/ES-100 to prevent the drug release in the stomach. The prepared microspheres were characterized for surface morphology, drug entrapment efficiency, drug loading, and *in vitro* drug release study. **Results and discussion:** The micromeritic studies showed that the prepared microspheres had improved flowability. The result obtained was found in the desired ranges, where percentage yield ranging from 65.75% to 67.46%, drug entrapment efficiency from 83.02% to 86.42%, and mean particle size ranges from 6.99 µm to 15.37 µm. Scanning electron microscopy permitted a surface topographical analysis. From the biodistribution study, it can be observed that AUC_{0-t} of the microspheres was 2.63-folds greater than the solution ($P < 0.05$) in the colon. **Conclusion:** The study reveals that drug release was significant at pH 7.4 from the microspheres at colon region, so the drug will be better absorbed in colon and can be used for successful treatment of the Crohn's disease and ulcerative colitis.

Key words: Biodistribution study, drug release studies, emulsification solvent diffusion, mesalamine, microspheres

INTRODUCTION

Both local and systemic deliveries of drugs can take place at the site of the colon through colon drug delivery system, and it can prevent the release of drug in gastric and small intestine region and affect an abrupt onset of drug release of drug soon after the entry of colon.[1] Colon-targeted mesalamine microspheres (MMS) have to retard the drug release in the stomach and small intestine and to ensure maximum drug release colonic environment with an improved patient compliance and low side effects.[2] The oral route is reflected to be most suitable for drug administration to the patients. Depending on the physicochemical properties of the drugs, most of the oral administered conventional dosages form normally dissolves in the stomach or intestinal fluid. It is a serious drawback in conditions where localized delivery of the drugs in the colon is required or in conditions where a drug needs to be protected from the hostile environment of the upper gastrointestinal tract (GIT). Dosage forms that deliver drugs into the colon rather than upper GIT offer a number of advantages.[3] Colon-targeted drug delivery would ensure direct treatment at the disease site, lower dosing, and less systemic side effects. In addition to restricted therapy, the colon can also be utilized as a portal for the entry of drugs into the systemic circulation. Mesalamine used to treat a certain bowel disease (ulcerative colitis). It helps to reduce symptoms of ulcerative colitis such as diarrhea, rectal bleeding, and stomach pain and used to decreasing the swelling in the colon. Free mesalamine undergoes rapid and nearly complete systemic absorption from

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the proximal intestine depending on concentration and local pH, followed by extensive metabolism.[4]

The objective of the study was to design colon-targeted MMS of retarding the drug release in the stomach and small intestine and to ensure maximum drug release in the physiological environment of the colon with enhanced patient compliance, lesser side effects, and most aspects of an ideal drug delivery system

MATERIALS AND METHODS

Mesalamine was procured from Albert David Ltd., Kolkata, India. Eudragit RS 100, Eudragit ES 100, chitosan, and xanthan gum were purchased form Merck Specialties Private Limited, Mumbai, India. All other chemicals were used as analytical grade. Preparation of MMS was done by emulsification solvent diffusion technique. Emulsification solvent diffusion technique was used to prepare the microspheres, which requires two immiscible phases such as internal and external phase containing a surfactant, which reduces the interfacial tension to form an emulsion. The required amount of mesalamine and selected polymers such as Eudragit RS 100, Eudragit ES 100, chitosan and xanthan gum were taken and dissolved separately in required volume of dichloromethane and isopropyl alcohol under sonication as mention in Table 1. The required amount of surfactant (polyvinyl alcohol) was dissolved in distilled water. The surfactant mixtures were permissible to cool at room temperature [Table 1]. The internal phase containing mesalamine and eudragit was added drop wise with the aid of 24-gauge syringe with stirring at a speed of 1500 rpm with mechanical homogenizer until the whole diffusion of the external phase takes place that is up to 8 h. Obtained microspheres were filtered and dried overnight at room temperature.[5-7]

Characterization of microspheres

Preformulation studies

A preformulation study was performed to ensure the development of a stable, therapeutically effective, and safe dosage form. Predictions of physicochemical properties of drug may finally confirm that no significant barriers are seen for the further development of formulation.[7-10]

API characterization

Bulk density

Bulk density or apparent density is defined as the ratio of mass of a powder to the bulk volume (Vo). The bulk density of a powder depends primarily on particle size distribution, particle shape, and tendency of the particles to adhere to one another.[11] Weigh accurately 25 g of drug sifted through 20# sieve and transferred in 100 ml graduated cylinder. Carefully

Table 1: Composition of mesalamine microsphere formulations

Ingredients drug: polymer ratio	Different formulation batches									
	MMS1	MMS2	MMS3	MMS4	MMS5	MMS6	MMS7	MMS8	MMS9	MMS10
	1:0.5:0.5	1:0.5:0.5	1:0.75:0.75	1:0.75:0.75	1:1:1	1:1:1	1:1.25:1.25	1:1.25:1.25	1:1.5:1.5	1:1.5:1.5
Internal phase										
Mesalamine (mg)	500	500	500	500	500	500	500	500	500	500
Eudragit RS 100 (mg)	250	–	375	–	500	–	625	–	750	–
Chitosan (mg)	250	–	375	–	500	–	625	–	750	–
Eudragit ES 100 (mg)	–	250	–	375	–	500	–	625	–	750
Xanthangum	–	250	–	375	–	500	–	625		750
Dichloromethane (ml)	10	10	15	15	20	20	25	25	30	30
Iso-propyl Alcohol (ml)	10	10	15	15	20	20	25	25	30	30
Dibutyl Pthalate (ml)	5	5	5	5	5	5	5	5	5	5
External phase										
PVA (mg)	50	50	75	75	100	100	125	125	150	150
Distilled water (ml)	75	75	100	100	125	125	150	150	175	175
Total quantity (mg)	1050	1050	1325	1325	1600	1600	1875	1875	2150	2150

MMS: Mesalamine microsphere , PVA: Polyvinyl alcohol, DCM: Dichloromethane, IPA: Isopropyl alcohol

Table 2: Flow properties data of the prepared microspheres

Fomulation code	Evaluation parameters				
	Angle of repose (θ)	Bulk density (g/cm³)	Tapped density (g/cm³)	Carr's index (%)	Hausners ratio
MMS 1	23.75	0.51±0.01	0.57±0.01	11.86	1.11
MMS 2	24.46	0.52±0.01	0.55±0.02	12.00	1.09
MMS 3	25.20	0.51±0.01	0.53±0.01	10.53	1.12
MMS 4	25.24	0.52±0.01	0.57±0.01	11.48	1.13
MMS 5	25.35	0.52±0.01	0.59±0.01	11.53	1.16
MMS 6	23.56	0.50±0.01	0.57±0.01	11.80	1.10
MMS 7	24.35	0.52±0.01	0.55±0.02	12.05	1.09
MMS 8	25.18	0.50±0.01	0.53±0.01	11.15	1.11
MMS 9	25.45	0.52±0.01	0.57±0.01	11.42	1.10
MMS 10	25.38	0.53±0.01	0.59±0.01	11.55	1.13

MMS: Mesalamine microsphere

Table 3: Selection of internal phase

Concentration of polymer in internal phase (mg)		Formation of microspheres		Physical appearance of microspheres		Particle size (in μm)	
RS 100 and chitosan	ES100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum
300 and 300	300 and 300	+	+	Irregular spherical	Irregular spherical	14.28	16.25
350 and 350	350 and 350	+	+	Spherical	Spherical	14.83	16.56
400 and 400	400 and 400	+	+	Spherical	Spherical	15.56	17.25
450 and 450	450 and 450	+	+	Spherical	Spherical	16.52	19.18
500 and 500	500 and 500	+	+	Spherical	Spherical	16.99	20
550 and 550	550 and 550	+	+	Irregular spherical microspheres	Irregular	17.35	2188
600 and 600	600 and 600	+	+	which collapses after some time	Irregular microspheres spherical microspheres which collapses after	19.6	24.17
650 and 650	650and 650	+	+	Spherical	some time	28.25	30.46
700 and 700	700and 700	+	+	Spherical	Spherical	33.16	533.89
750 and 750	750 and 750	+	+	Spherical rigid	Spherical rigid	35.8	36.42

level the powder without compacting, and read the unsettled apparent Vo. Calculate the appearance bulk density in g/ml by the following formula-1:

$$\text{Bulk density} = \frac{\text{Weight of the powder(M)}}{\text{Volume of the packing(VO)}} \quad (1)$$

Tapped density

The blend after determining the bulk density was subjected to mechanical tapping in the tapped density tester (USP I apparatus) that operates at a drop of 14 ± 2 mm at a nominal rate of 300 drops per minute. The cylinder was tapped 500 times initially and the tapped Vo was measured. The tapping was repeated for an additional 750 times and the tapped Vo was measured, V b. If the difference between the two Vos is <2%, Vb is the final tapped Vo, Vf.[12] It was repeated in increments of 1250 taps, as needed, until the difference between succeeding measurements is <2%. The tapped density was calculated, in g/ml, by the formula-2:

$$\text{Tapped density} = \frac{\text{Weight of the powder(M)}}{\text{Tapped Volume of the packing(Vf)}} \quad (2)$$

Hausner's ratio

Hausner's ratio gives an idea regarding the flow of the blend. It is the ratio of tapped density to the apparent density.[13] Hausner's ratio was calculated using the formula-3:

Table 4: Selection of concentration of polymer in the internal phase

Drug: Polymers ratio	Interna l phase (ml)	External phase (ml)	PVA (mg)	Drug content (%)		Free drug content (%)		% Entrapment	
				RS 100 and Chitosan	ES 100 and Xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthangum
01:01:01	20●	125	100	32.42	30.14	27.8	28.26	20.42	19.25
1:1:1	20▲	125	100	36.64	35.9	10.7	10.89	33.94	32.3
1:1:1	20*	125	100	28.75	27.09	32.65	32.2	17.15	16.1
1:1:1	20♦	125	100	36.48	35.55	9.86	10.85	35.32	34.5
1:1:1	20▲●	125	100	52.6	52.1	12.9	13.15	72.7	71.6
1:1:1	20♦▲	125	100	65.75	65	5.1	5.68	83.02	80.47
1:1:1	20♦●	125	100	36.48	35.35	9.56	10.85	35.32	34.5
1:1:1	20▲*	125	100	41.87	40.56	12.22	13.15	28.67	28.3
1:1:1	20♦*	125	100	50.15	49.35	15.7	17.8	29.3	25.75
1:1:1	20●*	125	100	18.3	17.38	22	22.45	25.6	24.1

●: Ethanol, ▲: Dichloromethane, ♦: IPA, *Methanol, PVA: Polyvinyl alcohol

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (3)$$

Compressibility index (CI)

The CI measures the propensity of powder to be compressed. The packing ability of drug was evaluated from change in Vo, which is due to rearrangement of packing occurring during tapping.[14] It is indicated as Carr's CI and can be calculated using formula-4:

$$\text{Compressibility index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} * 100 \quad (4)$$

Angle of repose

Irregular flow of powders from the hopper produces tablets with non-uniform weights. As a result, content uniformity and dose precision cannot be achieved in production of tablets and amp; capsules. The angle of repose (θ) is defined as the maximum angle that can be obtained between the freestanding surface of a powder heap and the horizontal plane and can be calculated by the formula-5:[15]

$$\text{Tan } \theta = \frac{\text{Height of pile (h)}}{\text{Bulk density radius of the base of pile (r)}} \quad (5)$$

Particle size analysis

The diameter of microspheres from each formulation was determined using an optical microscope. The samples were suspended in dispersion, and individual microsphere diameter was measured using micrometers. About the diameter of 500 microspheres was measured, and the mean particle diameter was calculated.[16]

Surface morphology

The samples for the scanning electron microscopy (SEM) analysis were prepared by sprinkling the microspheres on one side of an adhesive stub. Then, the microspheres were coated with gold before microscopy. Finally, the morphology and size of the microspheres were observed with the scanning electron microscope (FEI Quanta-200 MK2, Netherlands).[17]

Differential scanning calorimetry (DSC) studies

Compatibility studies were performed by DSC (Q10 V9.0 Build 275). The pure drug along with individual excipients was analyzed for DSC to know the compatibility of excipients with drug. DSC is used to determine the specific heat and enthalpies of transition. The area under the obtaining curve

Table 5: Selection of surfactant concentration in the external phase

Drug: polymer	PVA (mg)	External phase (water) (ml)	Physical appearance		Particle size in µm		Drug content (%)		% Entrapment		Free drug content	
			RS 100 and Chitosan	ES 100 and xanthangum	RS 100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and Chitosan	ES 100 and xanthan gum	RS100 and Chitosan	ES 100 and xanthan gum
1:1:1	50	125	Large clumps	Large clumps	-	-	-	32.22	-	-	-	-
1:1:1	75	125	Irregular large	Irregular large	16.86	18.56	35.5	65.00	81.5	80.2	7.30	8.60
1:1:1	100	125	Uniform spherical rigid	Uniform spherical	16.99	20	65.75	65.22	83.02	80.47	5.10	5.68
1:1:1	125	125	Uniform spherical rigid	Uniform spherical rigid	20.25	22.39	65.47	62.11	82.88	80.15	6.15	6.11
1:1:1	150	125	Uniform spherical rigid	Uniform spherical rigid	21.56	24.67	63.2	62.05	80.5	79.45	7.50	7.92
1:1:1	175	125	Uniform spherical rigid	Uniform spherical rigid	25.22	29.32	62.4	59.65	77.45	76.65	7.90	8.30
1:1:1	200	125	Uniform spherical rigid	Uniform spherical rigid	27.46	31.28	59.88	58.21	73.9	72.55	7.70	8.48
1:1:1	225	125	Uniform spherical rigid	Uniform spherical rigid	27.98	29.54	58.36	55.36	70.45	70.12	10.50	11.26
1:1:1	250	125	Irregular big	Irregular big	30.75	32.22	55.84	51.56	68.05	67	13.55	15.30
1:1:1	275	125	Irregular big	Irregular big	33.13	34.57	52.2		64.8	60.5	16.60	17.11

PVA: Polyvinyl alcohol

Table 6: Effect of external phase VO on microspheres

PVA (mg)	External phase (water) (ml)	Physical appearance		Particle size in (μm)		Drug content (%)		% Entrapment		Free drug content	
		RS100 and chitosan	ES100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and Xanthan gum
100	75	Irregular	Irregular	34.12	35.36	45.76		47.60	44.10	9.10	11.5
100	100	Spherical	Spherical	32.62	34.91	51.53	48.20	61.83	58.48	5.60	5.85
100	125	Spherical	Spherical	16.99	20.0	65.75	65.00	83.02	80.47	5.10	5.68
100	150	Spherical uniform	Spherical uniform	15.56	17.25	65.05	62.40	79.16	81.10	4.30	4.82
100	175	Spherical uniform	Spherical uniform	15.35	16.86	63.90	60.32	66.50	69.5	7.65	8.38
100	200	Spherical uniform	Spherical uniform	14.92	15.48	60.32	56.30	56.60	55.74	10.30	11.5
100	225	Spherical uniform	Spherical uniform	13.56	13.98	57.43	52.45	50.25	48.55	15.50	17.2
100	250	Irregular shape	Irregular shape	12.67	13.32	53.90	48.60	48.20	36.82	17.22	20.5
100	275	Irregular non uniform	Non uniform	12.56	13.12	48.20	42.78	37.90	33.15	18.60	23.1
100	300	Irregular large size	Irregular big large size	12.15	12.63	44.56	40.88	33.88	29.40	19.95	

PVA: Polyvinyl alcohol

Table 7: Effect of internal phase VO on microspheres

Internal phase (ml)	Particle size (in μm)		Drug content (%)		Free drug content (%)	% Entrapment
	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	ES 100 and xanthan gum	RS100 and chitosan
10	32.1	33.55	47.61	45.76	11.5	47.6
15	31.53	32.65	51.53	47.07	5.85	58.48
20	16.99	20.0	65.75	65	5.68	83.02
25	16.24	17.36	62.23	60.45	5.15	82.14
30	15.35	16.58	60.95	58.2	9.23	79

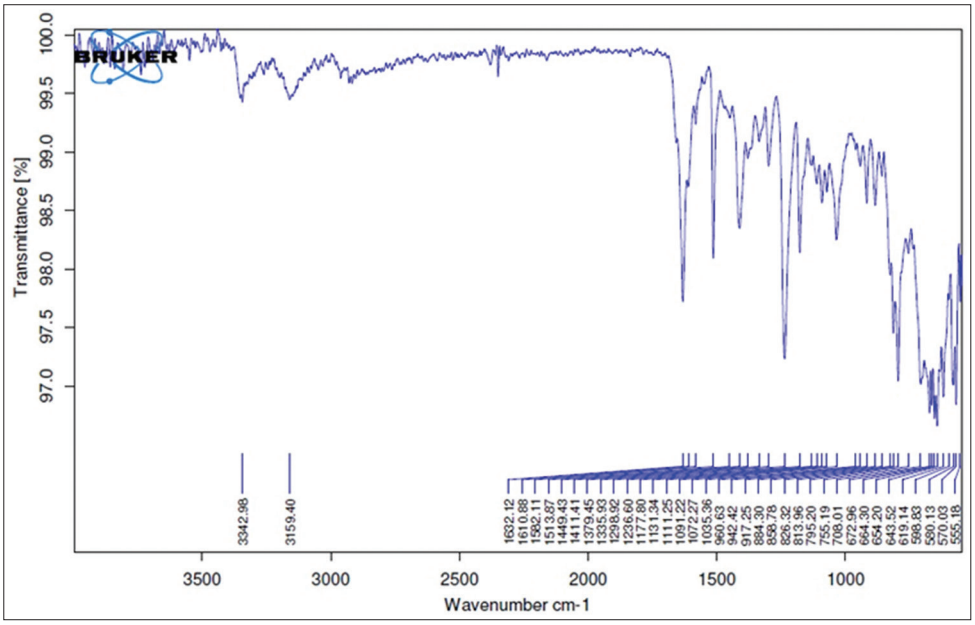

Figure 1: Fourier-transform infrared spectroscopy graph of mesalamine

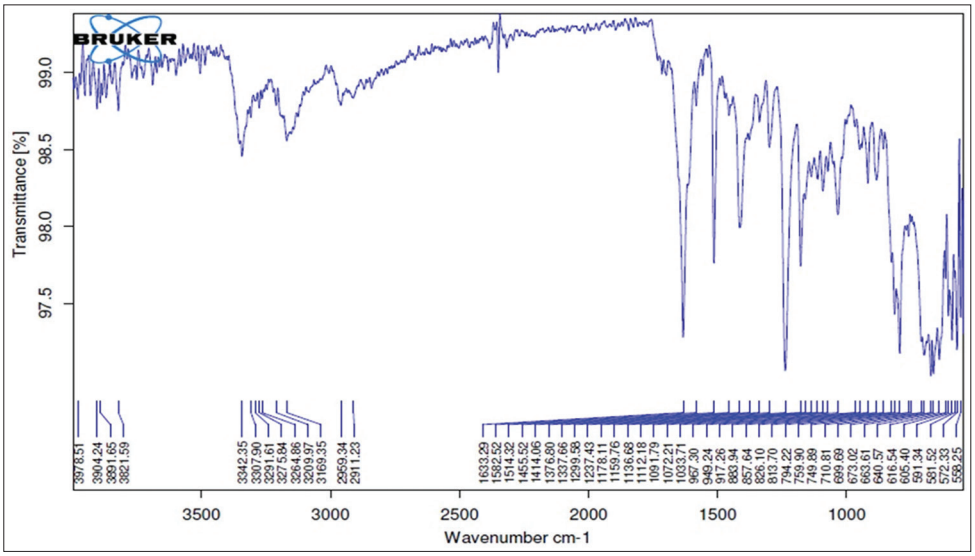

Figure 2: Fourier-transform infrared spectroscopy graph of mesalamine + Eudragit RS 100 and chitosan

is direct measure of the heat of transition. Thermograms were attained using a DSC at a heating rate 15°C/min over a temperature range of 0–1000°C. The sample was hermetically sealed in an atmosphere.[18]

In vivo biodistribution studies

All animal experiments were permitted by the experimental Animal Ethical Committee of Samskruti College of

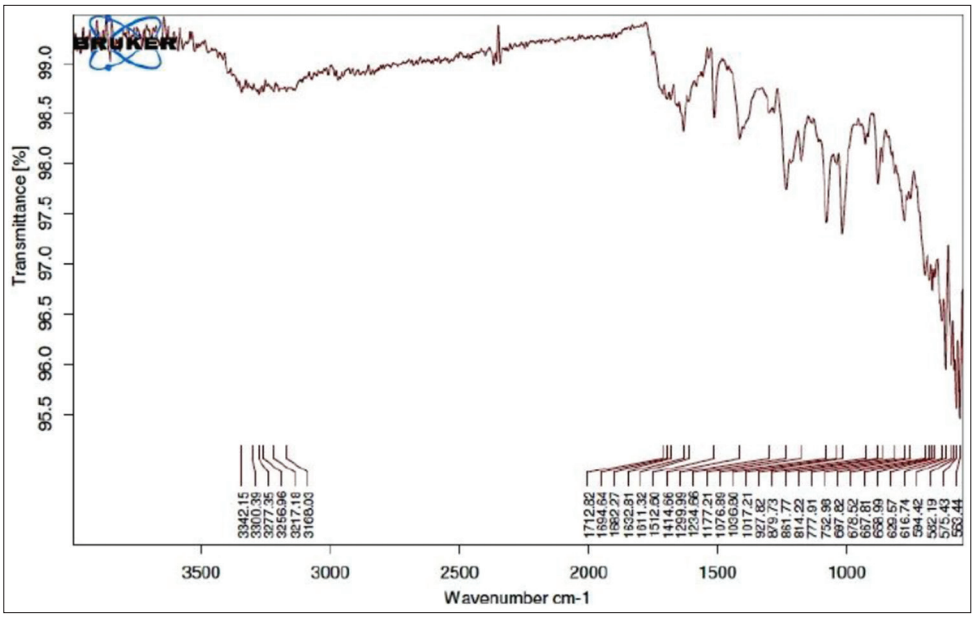

Figure 3: Fourier-transform infrared spectroscopy graph of optimized formulation

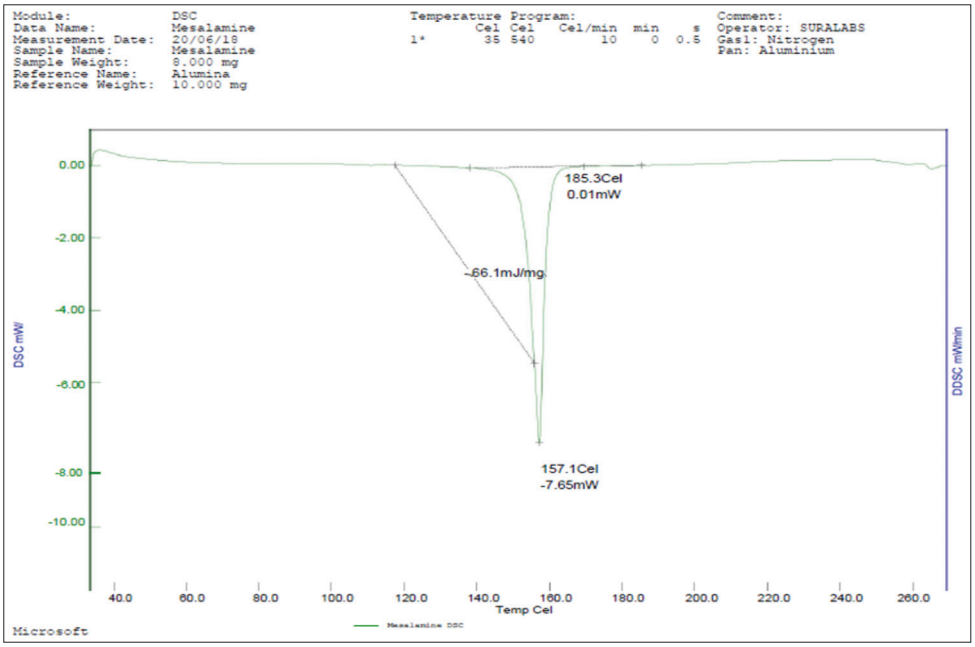

Figure 4: Differential scanning calorimetry curve of mesalamine

Pharmacy, Kondapur village, Ghatkesar Mandal, Medchal district, T.S., approval no-CPCSEA/IAE/EXP/29/409/2017/EXP/82. Before administration, the same weight of mice was selected for biodistribution studies, kept in well-spaced ventilated cages, and maintained on a normal diet (water *ad libitum*). The mesalamine-loaded microspheres or mesalamine solution was administered to mice by intragastric administration (10 mg/kg). 20 mice were divided randomly into two groups, each containing 10 mice. The MMS and mesalamine pure drug solution were orally administrated to the mice (10 mg/kg). At different time intervals soon after administration (1, 4, 8, 12, and 24 h), six mice in each group were picked up randomly. The stomach, small intestine, and colon were immediately removed, and approximately 100 mg of tissue slices were excised, weighed, and stored at −20°C until analysis.[19]

RESULTS AND DISCUSSION

MMS were successfully prepared by emulsion solvent evaporation technique. Composition of all formulations (MMS 1–MMS 10) of colon-targeted microspheres of mesalamine is shown in Table 1.

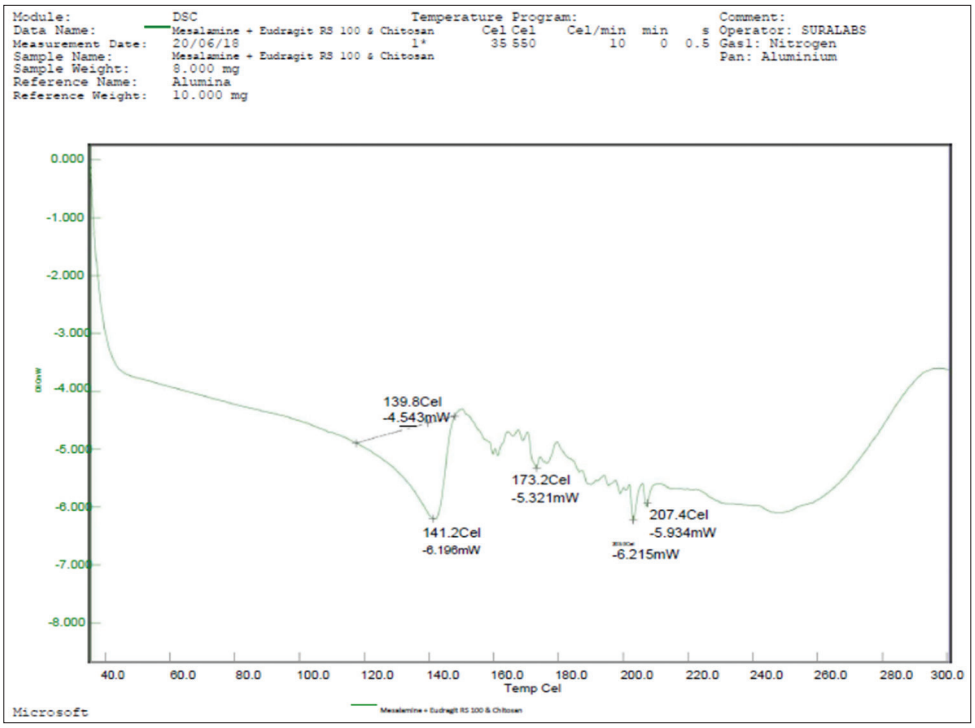

Figure 5: Differential scanning calorimetry curve of mesalamine + Eudragit RS 100 and chitosan

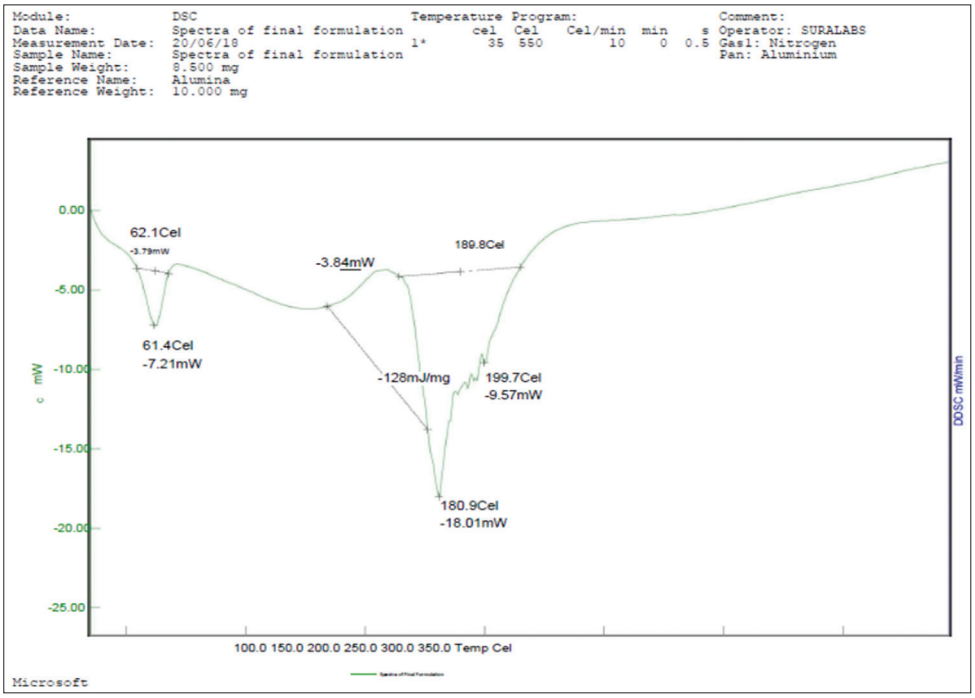

Figure 6: Differential scanning calorimetry curve of optimized formulation

Fourier-transform infrared spectroscopy (FT-IR) studies

The characteristic peaks of mesalamine are carboxylic acid stretch R-C=O-OH with peaks at 3342 and 1315 cm^{-1}, C=O stretch at 1789 cm^{-1}, -C_6H_5 aromatic ring stretch at 1645 cm^{-1}, –C-H (aromatic) stretch at 1450 cm^{-1}, and -C=C (aromatic) at 1490 cm^{-1}. Eudragit RS 100 [Figures 1-3] showed hydroxyl group stretching (-OH) at 2989 cm^{-1}, alkyl group (CH-R) stretching at 2997 cm^{-1}, the ester linkage (C=O-O-R) stretching at 1726 cm^{-1}, carboxylic acid (C=O-OH) stretching at 1708 cm^{-1}, alkyl group (CH-R) bending at 1386, 1448, and 1483 cm^{-1}, and carboxylic acid bending peaks at 1159, 1188, and 1263 cm^{-1}. The characteristic peaks of Eudragit ES 100 are hydroxyl group stretching (-OH) at 3494 cm^{-1}, alkyl group (CH-R) stretching at 2993 cm^{-1}, the

Table 8: Effect of rate of stirring on microspheres

Drug/ polymer	Time of Stirring in H (at 1500 rpm)	Physical appearance		Particle Size		Drug content		Free drug Content (%)		% Entrapment	
		RS 100and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and Chitosan	ES 100 and xanthangum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and Chitosan	ES 100 and Xanthan gum
1:1:1	21	Suspension filtered as such	Suspension filtered as such	-	-	-	-	-	-	-	-
1:1:1	# 2	Suspension filtered as such	Suspension filtered as such	-	-	-	-	-	-	-	-
1:1:1	4	irregular shape	irregular shape	45.25	48.8	58.5	55.12	8.1	9.55	65.49	62.5
1:1:1	66	spherical	spherical	32.68	35	65.47	65.22	2.95	2.78	75.16	71.1
1:1:1	88	spherical rigid	spherical rigid	16.99	20	65.75	65	5.1	5.68	83.02	80.47
1:1:1	110	spherical rigid	spherical rigid	15.3	55.86	66.47	64.22	4.85	5.26	83.85	81.8

Table 9: Effect of time of stirring on microspheres

Drug/ polymer	Time of stirring in H (at 1500 rpm)	Physical appearance		Particle size		Drug content (%)		Free drug content (%)		% Entrapment	
		RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum	RS 100 and chitosan	ES 100and xanthan gum	RS 100 and chitosan	ES 100 and xanthan gum
1:1:1	1	Suspension filtered as such	Suspension filtered as such	-	-	-	-	-	-	-	-
1:1:1	2	Suspension filtered as such	Suspension filtered as such	-	-	-	-	-	-	-	-
1:1:1	4	Irregular shape	Irregular shape	45.25	48.8	58.5	55.12	8.1	9.55	65.49	62.5
1:1:1	6	Spherical	Spherical	32.68	35	65.47	65.22	2.95	2.78	75.16	71.1
1:1:1	8	Spherical rigid	Spherical rigid	16.99	20	65.75	65	5.1	5.68	83.02	80.47
1:1:1	10	Spherical rigid	Spherical rigid	15.3	55.86	66.47	64.22	4.85	5.26	83.85	81.8

Table 10: Effect of drug/polymer ratio on physical properties of microspheres

Drug: polymer ratio	Production yield (%)		Mean particle size in (μm)		Drug content (%)		Free drug content (%)		% Entrapment	
	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum	RS100 and chitosan	ES 100 and xanthan gum
1:0.5:0.5	78	77.37	16.99	20	65.75	65	5.1	5.68	83.02	80.47
1:0.75:0.75	84.48	86.56	16.24	17.36	66.53	64.23	5.25	5.69	85.48	83.39
01:01:01	89.3	88.32	15.37	16.2	67.46	65.84	4.43	5.05	86.3	83.91

ester linkage (C=O-O-R) stretching at 1784 cm^{-1}, carboxylic acid (C=O-OH) stretching at 1724 cm^{-1}, alkyl group (CH-R) bending at 1384, 1448, and 1487 cm^{-1}, and carboxylic acid bending peaks at 1161, 1186, and 1261 cm^{-1}. The bending peaks of meta- and para-substituted benzene were observed at 811 cm^{-1}, and the R-NH_2 bending peak was observed at 686 cm^{-1}. FT-IR spectra of physical mixture of mesalamine with Eudragit RS 100 and Eudragit ES 100 showed characteristic peaks of carboxylic acid stretch R-C=O-OH, observed at 3409 and 1315 cm^{-1}, C=O stretch at 1789 cm^{-1}, -C_6H_5 aromatic ring stretch at 1645 cm^{-1}, –C-H (aromatic) stretch at 1452 cm^{-1}, and -C=C (aromatic) stretch at 1490 cm^{-1}. The above results confirmed the absence of drug interaction within the polymers.

DSC studies

DSC thermogram of pure drug, mesalamine [Figures 4-6], showed a characteristic exothermic peak at 294.52°C, which was within the range of melting point of mesalamine. Eudragit L-100 and Eudragit S-100 exhibited a similar exothermic peak at 239.84°C and 222.86°C, respectively. The observed melting point range was found to be in close proximity to the values reported. Mesalamine peak was found at 287.11°C in a physical mixture of mesalamine with Eudragit RS-100 and Eudragit ES-100, and a characteristic peak was observed at 289.11°C. This study confirmed that there was no interaction between the drug and polymers used.

X-ray diffraction (XRD) studies

The powder XRD curves are represented in Figure 7. The 2θ values from the powder XRD studies for mesalamine were found to be 14.956° and a sharp intense peak indicated the crystallinity of the drug. The 2θ value of Eudragit ES-100 was found to be 42.709°, indicating its crystalline nature. The 2θ value of Eudragit RS-100 was found to be 14.487° and confirmed its amorphous nature by a broad peak. A physical mixture of mesalamine with Eudragit S-100 showed a sharp intense peak at 15.167° and that of mesalamine with Eudragit ES-100 showed a sharp peak at 15.084°, indicating that the drug and the polymer existed in the crystalline state.

SEM studies

SEM of the formulations revealed that the surface morphology of the prepared microspheres was found to be spherical. The surface of the spheres was rough with abrasions on it as shown in Figure 8. The production yield ranged from 78% to 89.3% for RS100 and chitosan-based microspheres and 77.37% to 88.32% for ES 100 and xanthan gum-based microspheres. Particle size was found to be in the range of 15.37–16.99 μm with Eudragit RS 100 and chitosan microspheres and 16.20–20.0 μm with Eudragit ES 100 and xanthan gum microspheres. The percentage drug entrapment was found to be in the range of 64.23–67.46% for all

Table 11: Dissolution reading of the formulated batches with final selected properties

Medium	Time (h)	% Cumulative drug release									
		MMS1	MMS2	MMS3	MMS4	MMS5	MMS6	MMS7	MMS8	MMS9	MMS10
PH 1.2	1	0.02	0.034	0.0126	0.06	0.07	0.01	0.02	0.07	0.02	0.02
	2	0.03	0.05	0.26	0.06	0.08	0.02	0.03	0.08	0.03	0.03
PBS PH 6.8	3	0.12	0.17	0.11	0.17	0.19	0.1	0.12	0.015	0.12	0.12
	4	0.12	0.17	0.13	0.17	0.2	0.12	0.13	0.1	0.12	0.12
	5	15.14	15.09	17.44	17.8	19.6	21.48	24.83	21.76	20.1	19.22
	6	20.9	18.07	21.25	22.3	26.3	23.63	24.52	27.82	24.3	23.48
	7	20.49	22.4	28.09	25.88	36.8	33.95	33.14	35.9	32.99	33.6
PBS PH 7.4	8	36.19	34.17	41.44	39.38	45.7	39.23	48.92	39.55	43.45	38.66
	12	52.79	50.33	59.6	48.35	69.9	51.46	61.88	56.49	62.52	43.4
	16	65.46	52.42	70.28	57.9	67.8	66.15	70.2	60.44	68.88	56.1
	20	76.06	64.98	79.3	68.95	83.8	75.55	78.72	67.46	75.15	64.98
	24	79.9	73.43	81.88	79.1	92.1	87.82	83.26	75.35	79.4	71.99

MMS: Mesalamine microsphere

Table 12: The $AUC_{0-24\ h}$ of mesalamine in stomach, small intestine, and colon after intragastric administration of microspheres and solution to mice (n=6)

Formulation	Stomach	Small intestine	Colon
Solution (μg h/g), mean±SD	137.5±12.3	146.7±16.4	71.2±8.1
Microspheres (μg h/g), mean±SD	55.3±4.6	90.3±8.2	187.2±24.3
Ratio[a]	0.4	0.62	2.63*

The ratio was AUC (Microspheres)/AUC (Solution); *P<0.05 for microspheres versus solution. $AUC0-_{24\ h}$, Area under the plasma concentration–time curve from 0 to 24 h; SD: Standard deviation

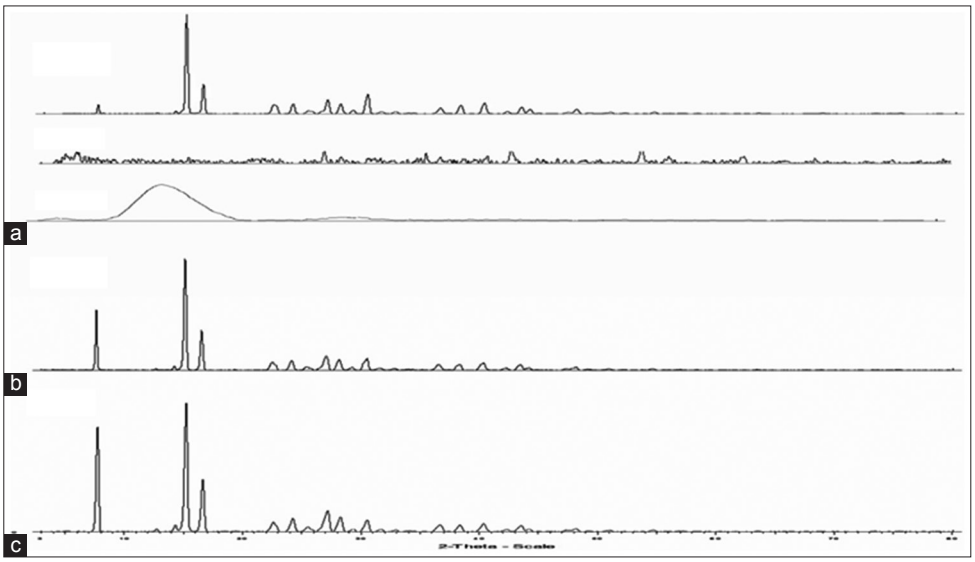

Figure 7: X-ray diffraction curves of (a) mesalamine; (b) Eudragit ES 100 and xanthan gum; (c) Eudragit RS 100 and chitosan; (d) mesalamine + Eudragit RS 100 and Chitosan; (e) mesalamine + Eudragit ES 100 and xanthan gum

formulations of Eudragit RS 100 or Eudragit RL 100 having chitosan and xanthan gum-based microspheres. Similarly, entrapment efficiency was found to be in the range of 80.47–86.30% for all formulations of Eudragit RS 100 or Eudragit RL 100 having chitosan and xanthan gum-based microspheres. The granular analysis of the prepared microspheres was performed and flow property was found to be best for formulation MMS 5 (25.35°) using Eudragit RS 100 and chitosan. Tap density was found to be in the range of 0.53–0.59 g/cm^3 for all the prepared microsphere formulations. The Carr's index was found to be in the range of 11.15–12.05% and Hausner's ratio was found to be in the range of 1.09–1.16

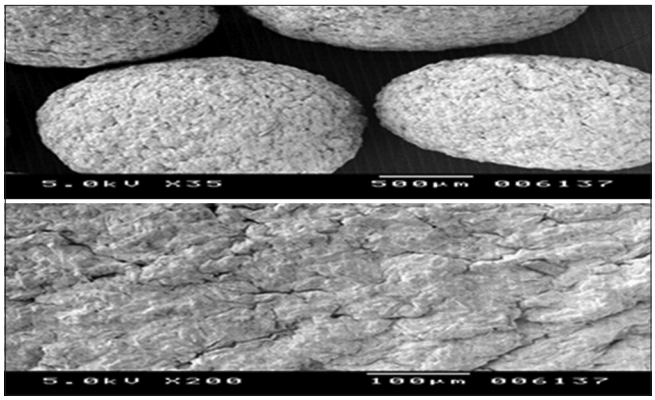

Figure 8: Scanning electron microscopy images of optimized formulation mesalamine microspheres 5 {drug:Eudragit RS-100:chitosan (1:1:1)}

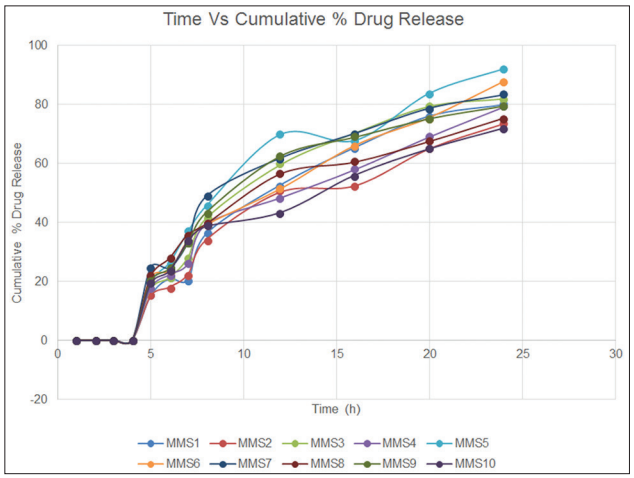

Figure 9: *In vitro* drug release profiles of different formulations

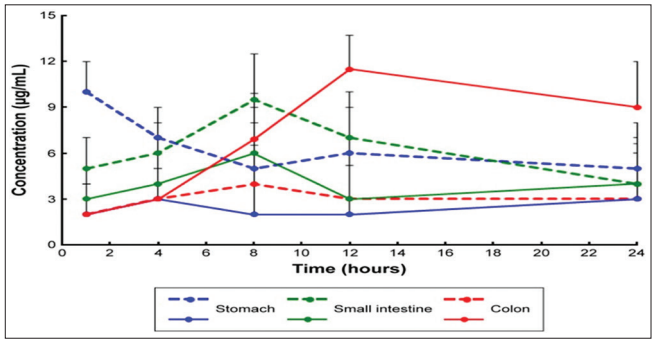

Figure 10: Distribution of drugs in tissues of mice following intragastric administration of a single 10 mg/kg dose of mesalamine-microspheres and mesalamine solution. Each point represents mean±SD of six mice. The solid lines indicate mesalamine-microspheres and the dotted lines indicate mesalamine solution. SD: Standard deviation

for all the microsphere formulations. The results are shown in Table 2. Selection of internal phase, concentration of polymer in the internal phase, surfactant concentration in the external phase, effects of external phase, internal phase Vo on microspheres, rate of stirring on microspheres, time of stirring on microspheres, and drug:polymer ratio on physical properties of microspheres are shown in Tables 3-10.

Table 13: Stability study data of the all batches for three months

Formulation code	Drug content (%)	
	After 30 days	After 90 days
MMS 1	79.71	79.54
MMS 2	73.35	73.20
MMS 3	81.68	81.56
MMS 4	79.00	78.92
MMS 5	92.08	92.00
MMS 6	87.74	87.65
MMS 7	83.20	83.05
MMS 8	75.15	75.00
MMS 9	79.30	79.19
MMS 10	71.90	71.76

MMS: Mesalamine microsphere

In vitro drug release studies

In vitro release studies were performed in USP type I apparatus (Basket Type) with stirring rate 50 rpm at 37±0.5°C. Initial drug release was performed for first 2 h in 900 ml of 0.1 N hydrochloric acid and next 2 h using dissolution media phosphate buffer pH 6.8 and remaining up to 24 h using phosphate buffer pH 7.4. Samples were withdrawn at regular intervals and analyze spectrophotometrically at 301.8, 330.8, and 330 nm, respectively, for calculations of the percentage of drug release. The formulation batches MMS 5 and MMS 6 show highest drug release, 84.50 and 82.40%, respectively [Table 11 and Figure 9].

In vivo biodistribution studies

Intragastric administration of optimized MMS and pure drug solution was given to the mice, and the reflects of tissue distribution as well AUC_{0-t} in stomach, intestine, and colon were estimated in 1, 4, 8, 12, and 24 h and the resulted values are shown in Table 12 and Figure 10. The results showed that the maximum drug concentration (9.6 µg/ml) was observed in the small intestine after 8 h and 3.1 µg/ml drug concentration in colon after 24 h of intragastric administration of pure drug solution [Figure 10], whereas in the optimized MMS, negligible amount of the drug was found in the stomach, small amounts were found in the small intestine, and a maximum percentage of micropheres was observed in the colon after 8 h of administration. Drug concentrations in the stomach, small intestine, and colon were significantly different between the microspheres and the drug solution. The AUC0–t of the microspheres was 2.63-fold higher compared to solution in colon ($P < 0.05$).

Stability studies

Stability studies of all the formulations (MMS 1–MMS 10) were performed as per the ICH and World Health Organization guideline that is with these following conditions such as temperature of 40°C ± 2°C and relative humidity of 75 ± 5% for 3 months for determination of drug content [Table 13].

CONCLUSION

Microspheres containing mesalamine were prepared by emulsion diffusion technique using Eudragit RS 100 and chitosan, Eudragit 100, and xanthan gum polymers. The drug content was uniform and reproducible in all the formulations. From the FT-IR spectral analysis showed that selected drug and polymers are compatible with out any interactions. Internal phase Vo: As there is increase in the internal phase Vo, there is decrease in size of the particles, content of drug, and entrapment efficiency and there is increase in the free drug content. Polymer concentration: As polymer concentration increases, the drug release decreases. Surfactant concentration: As surfactant concentration increases, there is increase in the particle size and decrease in the encapsulation efficiency and the production yield and larger microspheres. External phase Vo: As there is increase in the external phase Vo, there is decrease in drug content and drug entrapment and increase in the free drug content and particle size. Rate of stirring: If the stirring speed increases, there is increase in the free drug content and there is decrease in the drug content, entrapment efficiency, and particle size. Time of stirring: As stirring time increases, there is decrease in the free content of drug and size of the particle and there is increase in the entrapment efficiency and drug content. Drug:polymer ratio: As there is increase in the ratios, there is increase in the encapsulation efficiency and the production yield and decrease in the particle size. *In vitro* dissolution study showed that polymer concentration has played major role for controlling the drug release at the particular site of the G.I.tract. Among all the formulations, MMS 5 formulation was considered as stable and optimized batch based on maximum drug release charateristics on colonic environment.

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