"COLON-SPECIFIC CHRONO-DELIVERY OF MESALAZINE USING PULSINCAP TECHNOLOGY FOR ULCERATIVE COLITIS TREATMENT"

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ABSTRACT

The purpose of the present study was to design and evaluate an Oral, site specific, Pulsatile drug delivery system containing Mesalazine is an aminosalicylate drug used to treat mild to moderate active ulcerative colitis and also to maintain remission once achieved. The basic design consists of an insoluble hard gelatin capsule body, filled with powder blend and sealed with a hydrogel plug. The powder blend containing Mesalazine, Crospovidone, Lycoat, SSG, MCC and talc was prepared and evaluated for flow properties and FTIR studies. From the obtained results, F8 powder blend formulation was selected for further fabrication of pulsatile capsules. Hydrogel plug was formulated in a lone and in combination of hydrophobic polymer like ethyl cellulose with hydrophilic polymers like HPMC K15M as a ratio of Ethyl cellulose: HPMC K15M in 1:1, 1:2, and 2:1 ratio to maintain a suitable lag period and it was found that the drug release was controlled by the proportion of polymers used. The prepared formulations were evaluated for drug content, weight variation and *Invitro* release studies. FTIR studies confirmed that there was no interaction between drug and polymers and *Invitro* release studies of pulsatile device revealed that increasing hydrophilic polymer content resulted in delayed release of Mesalazine from the pulsincap after a predetermined lag time of 6hrs. Based on *invitro* studies performed, C3F8 was found to be optimized formulation.

Key words: Pulsatile system; time dependent delivery; Mesalazine; Chrono pharmaceutics; *In vitro* release studies.

INTRODUCTION:

Colon-specific drug delivery systems have gained considerable importance in the treatment of inflammatory bowel diseases such as ulcerative colitis, where localised drug action in the colon is essential for effective therapy. Mesalazine (5-aminosalicylic acid) is the drug of choice for the management of mild to moderate ulcerative colitis due to its topical anti-inflammatory effect on the colonic mucosa. However, conventional oral formulations of mesalazine often suffer from premature drug release and absorption in the upper gastrointestinal tract, leading to reduced drug availability at the site of inflammation and increased systemic exposure. [1,2]. To overcome these limitations, various colon-targeted delivery approaches, including polymeric systems, delayed-release formulations, and microbially triggered systems, have been explored to enhance colonic drug localisation and therapeutic efficacy [3-5].

Chrono-delivery systems are designed to release drugs after a predetermined lag time by utilizing the relatively constant transit time of the small intestine. Pulsincap technology is a well-established time-controlled delivery system that enables site-specific drug release in the colon. [6,7] The Pulsincap system consists of a water-insoluble capsule body sealed with a swellable hydrogel plug, which remains intact during transit through the stomach and small intestine. After the programmed lag period, the hydrogel plug swells and is expelled, resulting in rapid and pulsatile drug release [8]. Formulation studies have demonstrated that Pulsincap-based systems improve colonic targeting of mesalazine and minimize premature drug release [9,10]. Thus, colon-specific chrono-delivery of mesalazine using Pulsincap technology represents a promising approach for improving therapeutic outcomes, reducing systemic side effects, and enhancing patient compliance in ulcerative colitis treatment.

MATERIALS &METHODS:

PREFORMULATION STUDIES: It is one of the important prerequisites in development of any drug delivery system. It can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients.

Solubility: The solvents used are water and methanol. Solubility was determined by adding Mesalazine in small incremental amounts to a test tube containing a fixed quantity of different solvents. After each addition, the system was vigorously shaken and examined visually for any undissolved solute particles.

Drug-Excipient compatibility studies:

The FTIR spectra were recorded using an IR spectrophotometer (IR-Affinity-1, Shimadzu, Japan). The IR spectra for the samples were obtained by KBr disk method. The pellets of drug and potassium bromide were prepared by compressing the powders at 20 psi for 10 min on a KBr-press, and the spectra were scanned in the wave number range of 4000- 600 cm^{-1}. FTIR study was carried out on Mesalazine, a physical mixture of Mesalazine and the best formulation.

STANDARD CALIBRATION CURVE:

The standard calibration curve of Mesalazine was developed in different pH media, such as pH 1.2 and pH 6.8 phosphate buffer. Two buffers were selected in order to mimic the in-vivo conditions of the GIT.

RESULTS AND DISCUSSION

PREFORMULATION STUDIES:

Solubility: Mesalazine was found to be more soluble in 6.8 pH buffer when comparted to other buffers.

Table 1. Solubility studies of Mesalazine in various solvents

Solvent	Solubility (µg/mL)
0.1 N HCl	0.816
6.8pH buffer	0.911
7.4pH buffer	0.524
Water	0.065

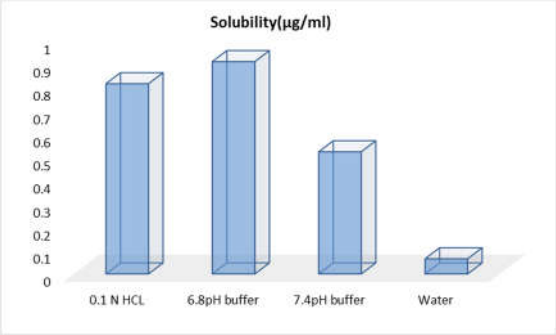

Figure 1. Solubility studies of Mesalazine in various solvents

Drug-Excipient compatibility studies: The IR spectrum of pure drug was found to be similar to the standard spectrum of Mesalazine. From the spectra of Mesalazine, the combination of Mesalazine with polymers, it was observed that all characteristic peaks of Mesalazine were

not altered and present without alteration in the combination spectrum, thus indicating compatibility of the drug and polymers. FTIR spectra of Mesalazine and the Optimised formulation are shown.

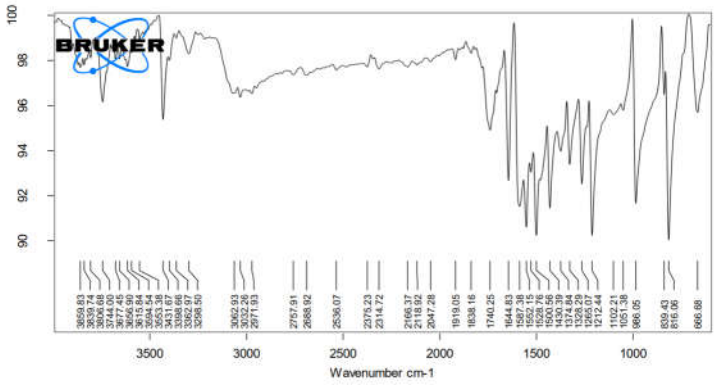

Figure 2. IR spectrum of pure Mesalazine

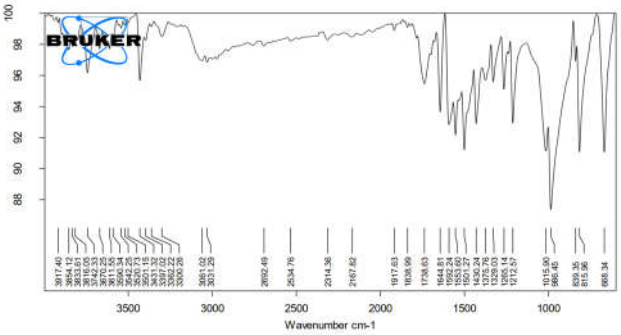

Figure 3. IR spectrum of Mesalazine Optimized Formulation

λ_{max} Determination of Mesalazine

The λ-max of Mesalazine of 100% solution i.e 12ppm (µg/ml) by using Single Beam Spectrophotometer (YIS-294) was found to be at 332 nm by using pH 6.8 phosphate buffer

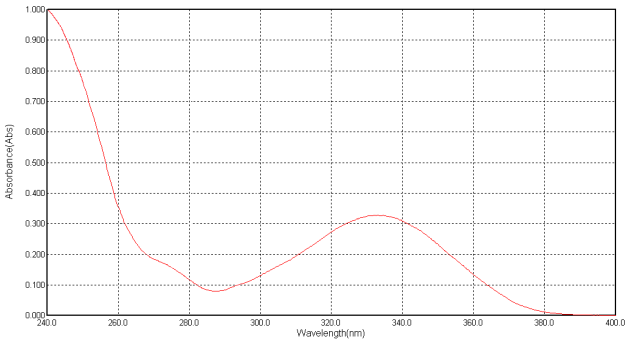

Figure 4. λ_{max} Determination of Mesalazine

Standard Calibration Curve:

Standard Calibration Curve in 1.2 pH:

Standard graph of Mesalazine showed linearity at the concentration range of 3-18µg in pH 1.2 at 332 nm with a correlation coefficient of 0.998.

Table 2. Data for the calibration curve of Mesalazine in pH 1.2 at 332 nm

Concentration (µg/mL)	Absorbance
0	0
3	0.098
6	0.212
9	0.305
12	0.417
15	0.545
18	0.645

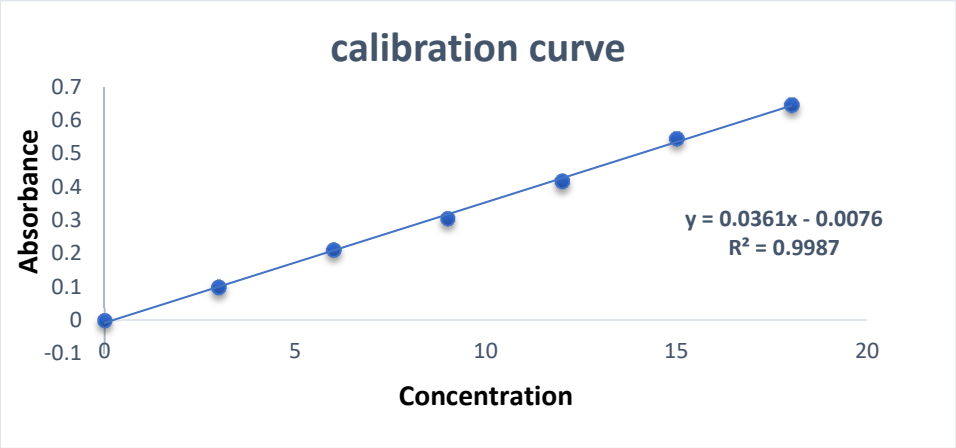

Figure 5. Standard Calibration Curve of Mesalazine in pH 1.2 at 332 nm

Standard Calibration Curve in 6.8 pH phosphate buffer:

Standard graph of Mesalazine in pH 6.8 phosphate buffer shows linearity in the concentration range of 3-18 µg with correlation coefficient of 0.999.

Table 3. Data for calibration curve of Mesalazine in pH 6.8 Buffer at 332 nm

Concentration (µg/mL)	Absorbance
0	0
3	0.115
6	0.219
9	0.325
12	0.437
15	0.545
18	0.655

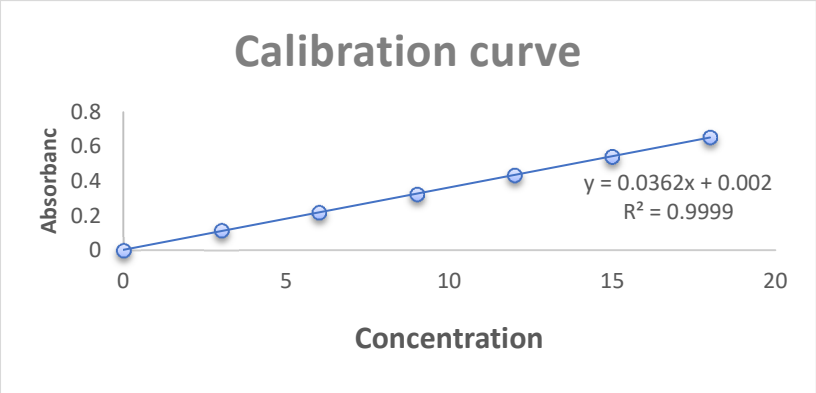

Figure 6. Standard Calibration Curve of Mesalazine in pH 6.8 Buffer at 332 nm

Flow properties of powder blend:

Table 4. Flow properties of powder blend

Formulation Code	Angle of Repose±SD	Bulk Density (g/ml)±SD	Tapped Density (g/ml)±SD	Carr's Index. (%)±SD	Hausner's ratio±SD
F1	28.47±1.48	0.327±0.002	0.445±0.003	18.24±0.14	1.19±0.47
F2	27.51±1.76	0.345±0.004	0.458±0.002	19.19±0.24	1.22±0.21
F3	26.36±1.15	0.359±0.003	0.469±0.001	16.45±0.39	1.21±0.35
F4	25.25±1.28	0.365±0.002	0.478±0.002	17.36±0.36	1.25±0.15
F5	27.58±1.36	0.337±0.001	0.454±0.001	18.19±0.42	1.19±0.24
F6	25.61±1.57	0.345±0.003	0.462±0.002	16.28±0.15	1.23±0.36
F7	24.89±1.25	0.359±0.002	0.474±0.001	18.42±0.19	1.21±0.24
F8	24.44±1.12	0.365±0.001	0.485±0.002	16.36±0.21	1.18±0.15
F9	26.61±1.32	0.348±0.003	0.457±0.003	17.25±0.19	1.17±0.12
F10	25.95±1.15	0.359±0.002	0.465±0.002	18.19±0.27	1.22±0.26
F11	24.24±1.50	0.369±0.002	0.475±0.001	19.42±0.36	1.21±0.17
F12	23.85±1.79	0.375±0.001	0.498±0.002	18.61±0.29	1.18±0.16

The angle of repose of different formulations was ≤ 29.59±0.25 which indicates that material had good flow property. So it was confirmed that the flow property of blends were free flowing. The bulk density of blend was found between 0.347±0.61 g/cm^3 to 0.484±0.78g/cm^3.Tapped density was found between 0.445±0.15g/cm^3 to 0.564±0.18 g/cm^3.These values indicate that the blends had good flow property. Carr's index for all the formulations was found to be between 16.28±0.15-19.42±0.36 and Hausner's ratio from 1.18±0.15-1.25±0.15 which reveals that the blends have good flow character.

Characterization of Tablets

Post Compression parameters

All the batches of tablet formulations were characterized for official evaluation parameters like Weight variation, Hardness, Friability, Tablet thickness and drug content and results are shown in the table.

Table 5. Characterization Mesalazine Tablets

Code	%Weight variation (mg)	Thickness (mm)	Diameter (mm)	Hardness	Friability (%)	Disintegrate time(sec)	Drug content (%)
F1	501.48 ±1.17	3.15±1.15	7.17±1.28	6.48±1.10	0.74±0.01	22±2	96.15 ±1.27
F2	502.21 ±1.28	3.39±1.38	7.34±1.45	6.65±1.25	0.68±0.02	18±2	97.24 ±1.45
F3	501.16 ±1.45	3.27±1.45	7.52±1.10	6.85±1.41	0.84±0.01	16±2	98.06 ±1.85
F4	499.34 ±1.37	3.48±1.02	7.69±1.35	6.96±1.36	0.46±0.02	11±2	96.76 ±1.35
F5	502.15 ±1.18	3.25±1.38	7.57±1.10	6.63±1.78	0.51±0.03	22±2	97.32 ±1.14
F6	499.19 ±1.52	3.39±1.10	7.12±1.28	6.74±1.15	0.72±0.01	17±2	98.35 ±1.69
F7	502.24 ±1.54	3.27±1.37	7.37±1.47	6.85±1.24	0.71±0.02	19±2	96.35 ±1.45
F8	501.35 ±1.27	3.59±1.18	7.76±1.15	7.15±1.51	0.76±0.01	16±2	97.48 ±1.78
F9	503.74 ±1.17	3.34±1.42	7.65±1.30	6.38±1.68	0.68±0.02	15±2	95.74 ±1.14
F10	499.36 ±1.20	3.21±1.18	7.45±1.75	6.57±1.52	0.42±0.01	16±2	96.25 ±1.02
F11	501.03 ±1.37	3.46±1.47	7.35±1.12	6.62±1.12	0.49±0.01	14±2	97.65 ±1.32
F12	500.26 ±1.54	3.51±1.20	7.69±1.79	6.98±1.37	0.53±0.02	14±2	99.34 ±1.69

Hardness of the tablet was acceptable and uniform from batch to batch variation, which was found to be 3.15±1.15-3.51±1.20kg/cm^2.All the formulations passed the weight variation test as the % weight variation was within the pharmacopoeia limits of the tablet weight. Friability values were found to be less than 1% in all the formulations F1–F12 and considered to be satisfactory ensuring that all the formulations are mechanically stable. The drug content values for all the formulations (F1-F12) was found to be in the range of 95.74±1.14-99.34±1.69%.

Dissolution studies of the tablets:

The prepared tablets were subjected to dissolution studies in order to know the amount drug release.

Table 6. % Cumulative drug release of formulations F1-F12

Time (mins)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0	0	**0**	0	0	0	0
5	28.73 ±1.27	24.85 ±1.85	41.42 ±1.12	55.15 ±1.84	17.21 ±1.45	23.26 ±1.69	45.24 ±1.57	**49.36 ±1.20**	25.48 ±1.95	32.08 ±1.21	42.89 ±1.45	50.75 ±1.17
10	32.85 ±1.49	36.42 ±1.49	56.21 ±1.37	68.48 ±1.52	35.23 ±1.78	37.15 ±1.52	54.51 ±1.85	**61.26 ±1.42**	38.82 ±1.74	45.48 ±1.51	54.24 ±1.20	64.26 ±1.25
15	41.74 ±1.18	47.35 ±1.20	67.36 ±1.27	76.18 ±1.36	42.15 ±1.20	45.42 ±1.45	65.28 ±1.45	**75.12 ±1.18**	45.45 ±1.15	58.05 ±1.45	66.42 ±1.10	73.12 ±1.67
20	52.47 ±1.38	58.63 ±1.41	75.75 ±1.69	85.61 ±1.17	55.42 ±1.36	57.36 ±1.02	78.42 ±1.21	**81.36 ±1.41**	59.21 ±1.52	67.78 ±1.96	77.19 ±1.74	86.36 ±1.41
30	65.81 ±1.45	68.49 ±1.35	89.15 ±1.14	93.25 ±1.20	62.26 ±1.51	64.25 ±1.36	83.26 ±1.56	**88.14 ±1.26**	64.48 ±1.36	76.36 ±1.24	88.46 ±1.17	92.14 ±1.85
40	76.84 ±1.82	74.31 ±1.18	91.28 ±1.22	98.47 ±1.09	71.24 ±1.54	72.15 ±1.18	94.19 ±1.86	**99.14 ±1.54**	78.82 ±1.45	87.09 ±1.76	91.78 ±1.52	98.58 ±1.59
50	86.24 ±1.91	86.53 ±1.69	98.19 ±1.69		84.62 ±1.74	85.26 ±1.75	98.24 ±1.10		87.49 ±1.84	95.31 ±1.52	98.53 ±1.45	
60	98.21 ±1.37	98.56 ±1.75			97.25 ±1.18	98.84 ±1.20			98.46 ±1.75	99.75 ±1.98		

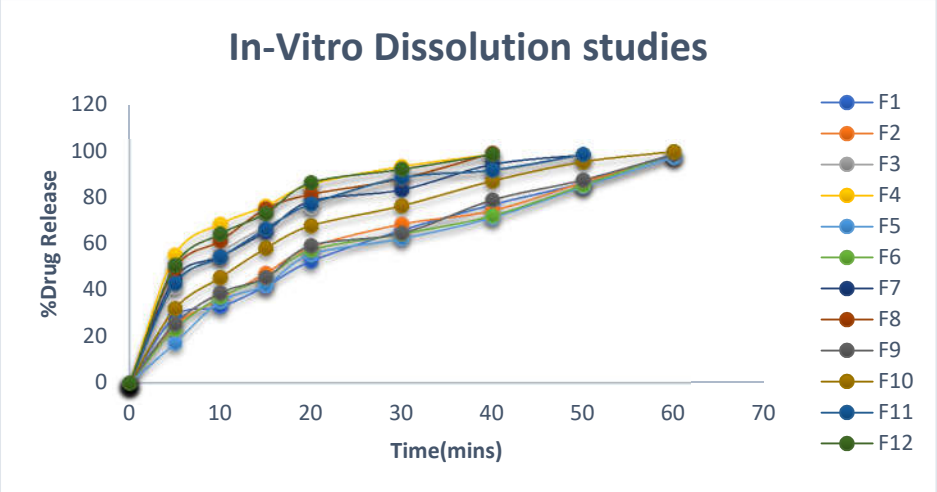

Figure 7. In vitro drug release of formulations F1-F12

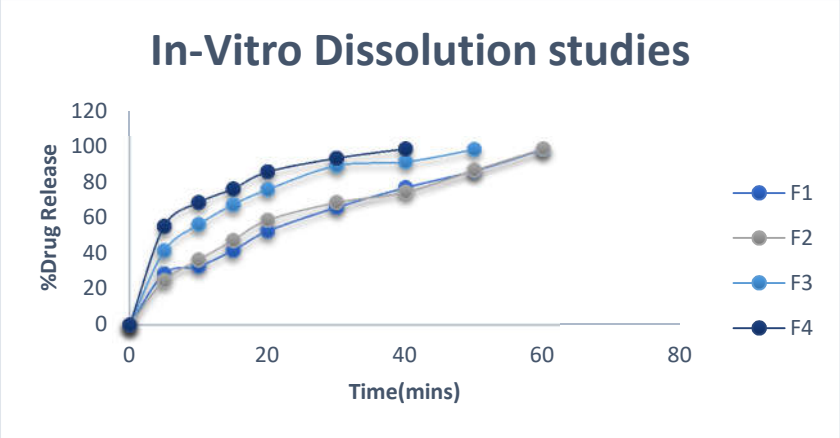

Figure 8. In vitro drug release of formulations F1-F4

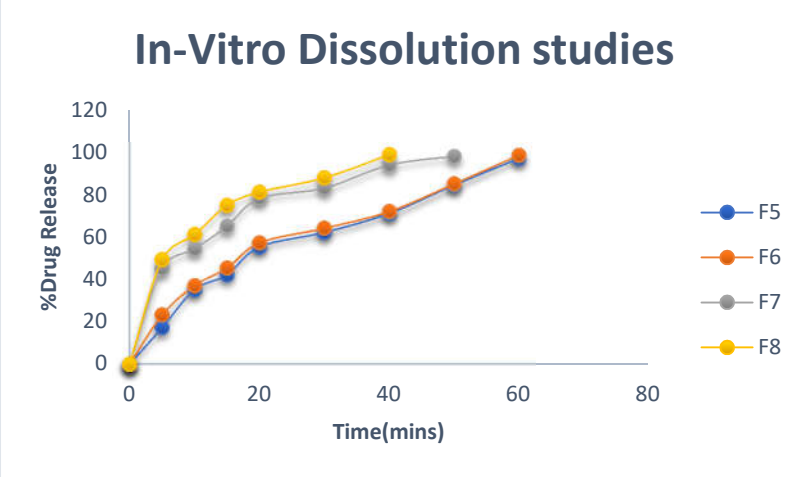

Figure 9. In vitro drug release of formulations F5-F8

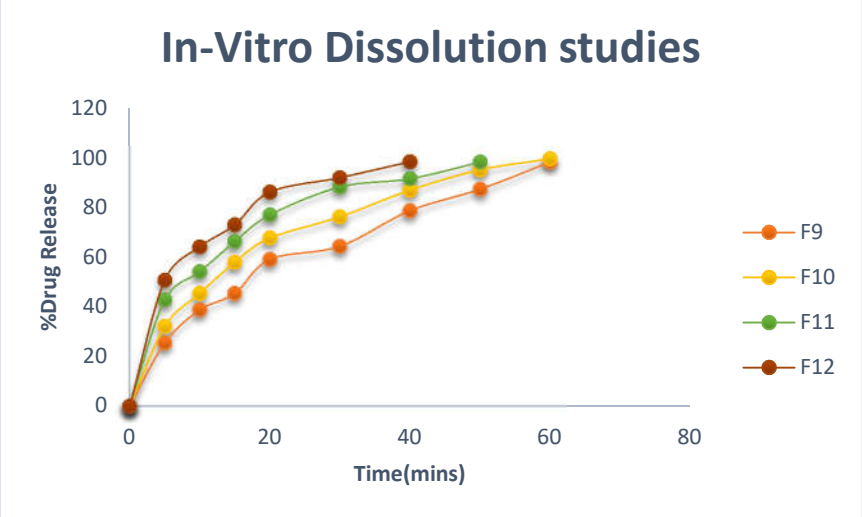

Figure 10. In vitro drug release of formulations F9-F12

From the in vitro drug release in studies, it was observed that the formulations containing Lycoat as a super disintegrant in different concentrations like 50mg, 75mg, 100mg, 125mg, reveals that the increased in the super disintegrant concentration decreases the drug release time and the F4 formulation containing Lycoat 125 mg concentration shows maximum amount of drug release (99.14±1.54%) at the end of 40mins.

So, F8 formulation containing 125mg in concentration of Lycoat shows max. release 99.14±1.54% within 40mins so that it is chosen as optimized formulation.

EVALUATION OF FORMALDEHYDE TREATED CAPSULES:

Physical tests:

Identification attributes: The size '0' capsules chosen were opaque, with white colored body and red cap. The normal capsule bodies were soft and sticky when touched with wet hand. After treating with formaldehyde, there were no significant changes in the physical appearance of the capsules except for the stickiness. The body of capsule was hard and non-sticking even when touched with wet hand due to treatment with the formaldehyde.

Visual defects: Among 100 capsules body which were treated with formaldehyde, about 15 to 20 capsule bodies showed visual defects. They were found to be shrunk and distortion into different shapes due to the complete loss of moisture.

Dimensions: Dimensional examination was done by using vernier calipers.

Average capsule length:

Before formaldehyde treatment (untreated cap and body) : 23.4 mm

After formaldehyde treatment(treated body and untreated cap): 22.2 mm

Average diameter of capsule body:

Before formaldehyde treatment : 8.7 mm

After formaldehyde treatment : 7.6 mm

Average length of capsule body:

Before formaldehyde treatment : 19.5 mm

After formaldehyde treatment : 18.4 mm

Discussion: On formaldehyde treatment, the "0" size capsules bodies showed a significant decrease in length and diameter and attained hardness.

Chemical test:

Qualitative test for free formaldehyde: The formaldehyde treated capsules were tested for the presence of free formaldehyde by comparing color of sample solution with standard

solution. It was found that the sample solution was not more intensity colored than the standard solution inferring that less than 20µg/ml of free formaldehyde was present in 25 capsule bodies.

Discussion: Limit test for the presence of residual formaldehyde, indicated that the amount of formaldehyde present in treated capsules was well within limits.

Optimization of formaldehyde treated capsule bodies exposed at various time intervals viz., 2, 4, 6, 8, 10hrs:

Table 7. Disintegration test for Treated Capsules

Capsule Code	Disintegration Time (hrs)	
	1.2 pH (2hrs)	6.8 pH (up to 24hrs)
(2rd hr)	2	_
(4th hr)	2	1
(6th hr)	2	7
(8th hr)	2	9
(10th hr)	2	12

Basing on the disintegration studies, it was observed that the 3rd capsule 6th hr treated capsule remained intact for 7 hrs so lag time was maintained. 4th and 5th remain intact for 9, 12 hrs respectively and therefore they were not selected for the formulation because the required lag time was 6hrs. As the required lag time is 6hrs, (6th hr treated capsule) was selected as optimized time for formaldehyde treatment for further studies.

In vitro release studies:

Dissolution study was carried out to measure the release rate of drug from prepared pulsincap formulation using USP I dissolution apparatus at 37^0C using 2 different dissolution media of pH 1.2, pH 6.8 phosphate buffer in order to mimic in vivo GIT conditions. Initially first 2hrs of dissolution was conducted in pH 1.2 buffer, followed by 10hrs of dissolution study in pH 6.8 phosphate buffer.

Table 8. *In vitro* dissolution data of formulations C1F8 to C5F8

Time (hrs)	C1F8	C2F8	C3F8	C4F8	C5F8
0	0	0	**0**	0	0
1	0	0	**0**	0	0
2	0	0	**0**	0	0
3	0.59±1.74	0.74±1.57	**0.98±1.48**	1.48±1.45	1.85±1.26
4	1.58±1.29	1.97±1.15	**2.18±1.67**	2.25±1.78	2.79±1.74
5	75.78±1.45	2.26±1.36	**2.85±1.50**	86.45±1.51	3.24±1.25
6	85.25±1.37	75.24±1.48	**89.24±1.48**	88.26±1.58	85.36±1.06
7	98.28±1.57	86.48±1.51	**94.54±1.65**	98.85±1.48	91.48±1.58

8		98.52±1.28	**99.68±1.48**		98.25±1.46
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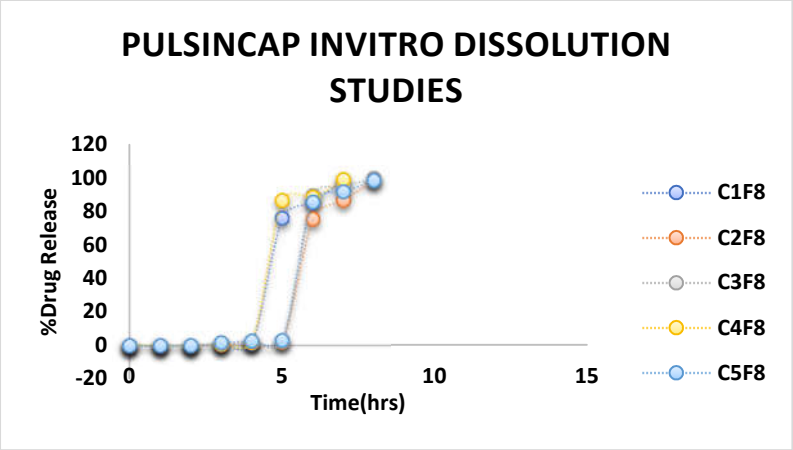

Figure 11. Dissolution plots for formulations C1F8 to C5F8

All the 5 formulations of Mesalazine pulsincaps were subjected to dissolution studies. Formulations C1F8, C2F8, C3F8, C4F8 & C5F8, contain the hydrogel plug with alone and combination of hydrophobic polymer and Hydrophilic polymer i.e., Lactose: HPMC in the ratio of 1:1, 2:1 & 1:2 of total 100mg weight of the plug.

It was observed that a proper lag time of 6 hours was maintained with minimal upper GIT drug release for the combination of Lactose and HPMC K15M hydrogel plug in the 2:1. It was observed that as the concentration of Hydrophilic polymer was increased the release rate of drug was delayed and finally burst release of drug from the formulation occurred after lag time. So, basing on these observations, of all the 5 pulsincap formulations, C3F8 formulation containing hydrogel plug of Lactose & HPMC K15M in 2:1 ratio was selected as optimized pulsincap formulation.

RELEASE KINETICS:

Dissolution data was fitted in Zero order, First order, Higuchi's and koresmayer peppas equations. The regression coefficient "R" values for zero order, first order, higuchi's and peppas for formulation C3F8 was found to be 0.620, 0.460, 0.417, and 0.821 respectively.

Table 9. Correlation coefficient "R" values of C3F8 optimized formulation

Models	R values
Zero order	0.707
First order	0.658

Higuchi	0.502
Koresmayer peppas	0.647
Peppas 'n'	2.190

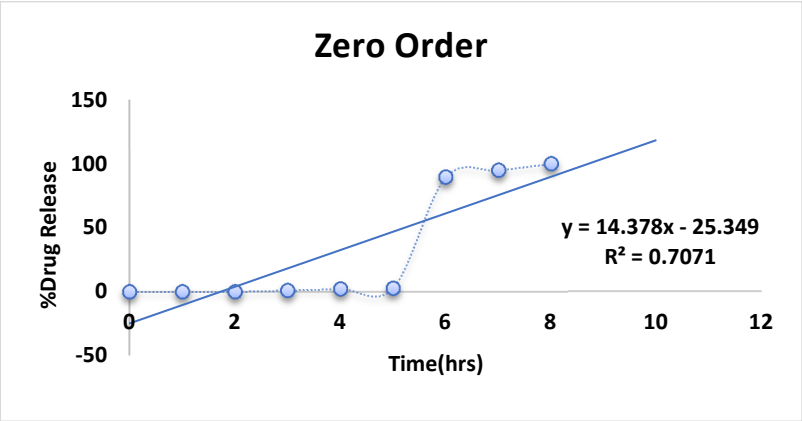

Figure 12. Zero order plot for optimized formulation C3F8

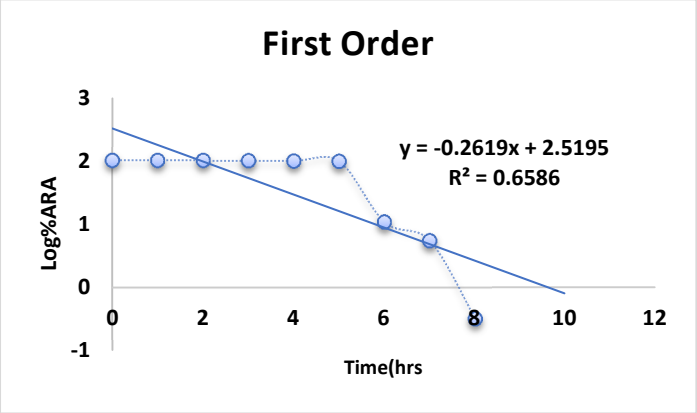

Figure 13. First order plot for optimized formulation C3F8

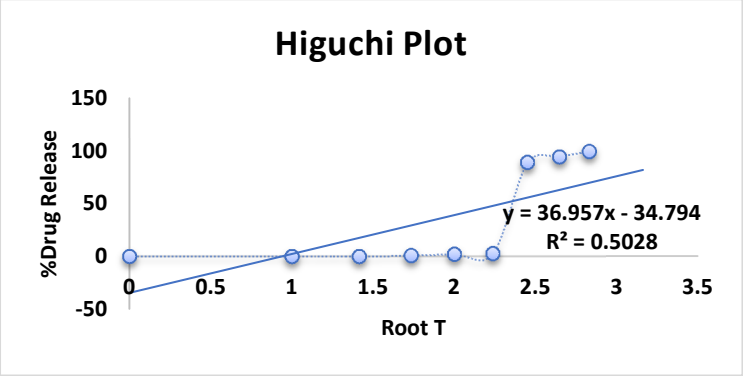

Figure 14. Higuchi's order plot for optimized formulation C3F8

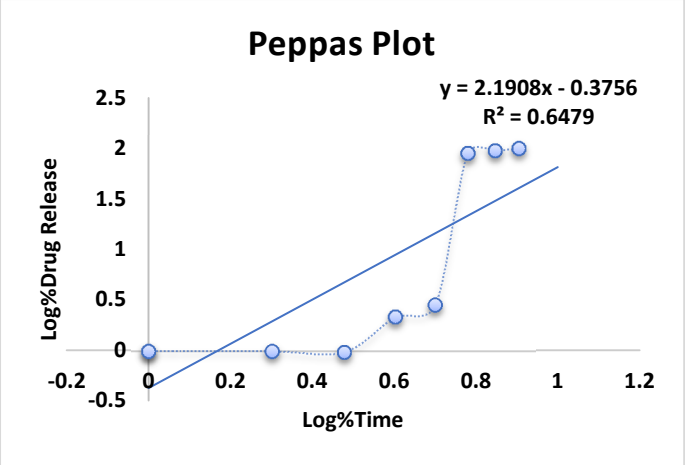

Figure 15. Koresmayer peppas order plot for optimized formulation C3F8

To analyze the mechanism of drug release from optimized C3F8 pulsincap formulation, data obtained from the drug release studies was subjected to different kinetic treatments. The correlation coefficient (R) was used as indicator of the best fitting for each of the models considered. The drug release kinetics for the optimized formulation C3F8 followed the Zero order and follows super case II transport mechanism.

CONCLUSION:

Pulsincap technology enables colon-targeted, time-specific delivery of Mesalazine for chronotherapeutic treatment. Preformulation studies confirmed drug-excipient compatibility, and formaldehyde-treated capsules remained intact for 24 hours, suitable for colon targeting. Hydrogel plugs of Ethyl Cellulose and HPMC K15M controlled lag time, with a 2:1 ratio achieving a 6-hour delay and burst release. Powder blends showed good flow and uniformity, and in vitro studies confirmed controlled release. Overall, this system is a promising approach for designing pulsatile, chronotherapeutic drug delivery formulations with precise time- and site-specific release.

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