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RESEARCH ARTICLE

# FORMULATION AND EVALUATION OF COLON TARGETED TABLETS OF MESALAZINE

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**ABSTRACT**

In the present investigation an attempt was made to formulate the time and pH dependent drug delivery system, reduce the frequency of dose administeration, to prevent ulcerative colitis by developing sustained delayed release tablets of Mesalazine using combination of Eudragit S-100 and L-100 as enteric coating. The core tablets of Mesalazine were prepared using wet granulation containing a superdisintegrant. The aim of present study is to develop colon specific drug delivery of Mesalazine sustained release matrix tablets for ulcerative colitis using HPMC K-4M and HPMC K-15M as a semisynthetic polymer. Effect of polymer concentration and superdisintegrant level was also investigated. The matrix tablets of Mesalazine are subjected to an *in-vitro* drug release study using simulated gastric fluid (0.1N HCl) for 2 hours, simulated intestinal fluid (pH 7.4) for 3 hours and simulated colonic fluid (pH 6.8) for 7 hours as dissolution fluid. The study showed that, lag time prior to drug release was highly affected by the coating. Colon drug delivery is advantageous in treatment of colonic disease and oral delivery of drugs that are unstable and susceptible to enzymatic degradation in upper GI tract. The disintegration data obtained from tablets demonstrated that disintegration data rate of studied tablets is dependent on: (i) The polymer used to coat the tablets (ii) pH of disintegration media. Results also demonstrated that combination of Eudragit S-100 and L-100 can be successfully used to coat tablets for colon targeted delivery of drug.

**Keywords:** Time and pH dependent drug delivery system, ulcerative colitis, mesalazine, wet granulation.

## INTRODUCTION

Over the past several years there has been a dramatic increase in bowel diseases. Approximately one third of patients with mucosal ulcerative colitis undergo operative treatment.[1] In recent years, colon targeted delivery systems have been the focus point of formulation laboratories because the colon is considered as a suitable site for the delivery of both conventional and labile molecules, and it is also a site for some specific diseases, such as, ulcerative colitis, crohn's disease, bowel cancer, some infections, and constipation, which require local delivery of the drug(s).[2] Colonic drug delivery may be achieved by either oral or rectal administration. Rectal dosage forms (enemas and suppositories), are not always much effective due to high variability in the distribution of drug administered by this route.[3] The major obstacle with the delivery of drugs by oral route to the colon is the absorption and degradation of the drug in the upper part of gastrointestinal tract (GIT) which must be overcome for successful colonic drug delivery.[4]

Various approaches have been used for oral delivery of drug(s) to the colon which includes time dependent delivery, pH- dependent systems and bacteria- dependent delivery. The pH dependent systems exploit the generally accepted view that pH of human gastrointestinal (GI) tract increases progressively from the stomach (pH 2-3), small intestine (pH 6.5-7) to the colon (7.0-8.0). Taking advantage of the highest pH value of the colon content, the dosage form containing the active drug in the core is coated with pH dependent material which dissolves at the pH of colon. But recent studies using sensitive and reliable equipments contradict the traditional view and provide evidence of a fall in pH at the GI region between ileum and colon.[5,6] Apparently, colon has a lower pH value (6.5) than the small intestine (7.0-7.8), and the jejunal region of some individuals has a higher pH range (6.1-7.2) than the small intestine or colon of other individuals.[2]

Ulcerative colitis is the anti inflammatory disease of the colonic mucosa which is restricted to large intestine and is usually treated with salicylates or glucocorticoids. However, during periods of remission mesalamine is the drug of choice. In this case it is desirable to localize the release of mesalamine to the afflicted site in the colon.[7-9] Thus, Mesalamine was used as a model drug in the present study. Mesalamine is an anti inflammatory drug, for oral adminsteration in the treatment of diseases of colon (ulcerative colitis, crohn's disease, carcinomas and infections) whereby high local concentration can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT or unnecessary systemic absorption.[10]

## OBJECTIVES

The objective of this study is to develop formulations using a combination of time and pH dependent system for delivering mesalamine to the colon and to sustain the release of the drug using various grades of HPMC (HPMC K-4M and HPMC K-15M) so as to reduce the dosing frequency of the drug and to demonstrate its site specificity in the colon. Effect of polymer concentration and the superdisintegrant concentration was also investigated.

Methacrylic acid copolymers such as Eudragit S-100 and Eudragit L-100 have been commonly used as pH

 

dependent polymers for coating solid dosage forms (because of their solubility at pH 6.0 or higher and 7.0 or higher respectively), none of them is suitable for use alone for coating of solid dosage forms that would start releasing the drug specifically at pH 6.4 which is generally considered as the suitable pH for colon targeted drug delivery.[11-13] A major drawback of Eudragit coated pH dependent formulation is premature release of drug in small intestine.[14]

## MATERIALS AND METHODS

Mesalazine (5-amino salicylic acid) was obtained from Hi-media Pvt Ltd., Mumbai. HPMC K-4M and HPMC K-15M were obtained as free gift sample from Colorcon Asia Pvt. Ltd., Goa. Crosspovidone, Eudragit L-100 and S-100 were obtained from Yarrowchem products, Mumbai. Starch and magnesium stearate were obtained from S.d fine chem., Mumbai and Lactose was obtained from Qualikems fine chemicals Ltd, New Delhi. All reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

## 1) ANALYTICAL METHOD VALIDATION

### 1.1. Linearity and Range[15]

Aliquots of different concentration of Mesalazine were prepared upto highest concentration, till linearity was observed and absorbance was recorded at 302 nm for acidic media, 331.60 nm and 331.70 nm for phosphate buffer of pH 6.8 and 7.4 respectively.

### 1.2. Limit of Detection (LOD)[15,16]

The LOD of Mesalazine was determined in placebo solution. It was based on the standard deviation of the blank. It was computed from the calibration graphs using the equation 3.3σ/s where σ is the standard deviation of three blank determinations and s is the slope of calibration curve. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

### 1.3. Limit of Quantitation[15,16]

The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. It was computed from the calibration graphs using the equation 10σ/s, where σ is the standard deviation of three blank determinations and s is the slope of calibration curve.

## 2) FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) STUDY

The IR absorption spectrum of the pure drug was taken in the range of 4000-450 cm using KBr pellet method .The major peaks were reported for evaluation of purity. Observed peaks are similar to reported peaks of Mesalazine.

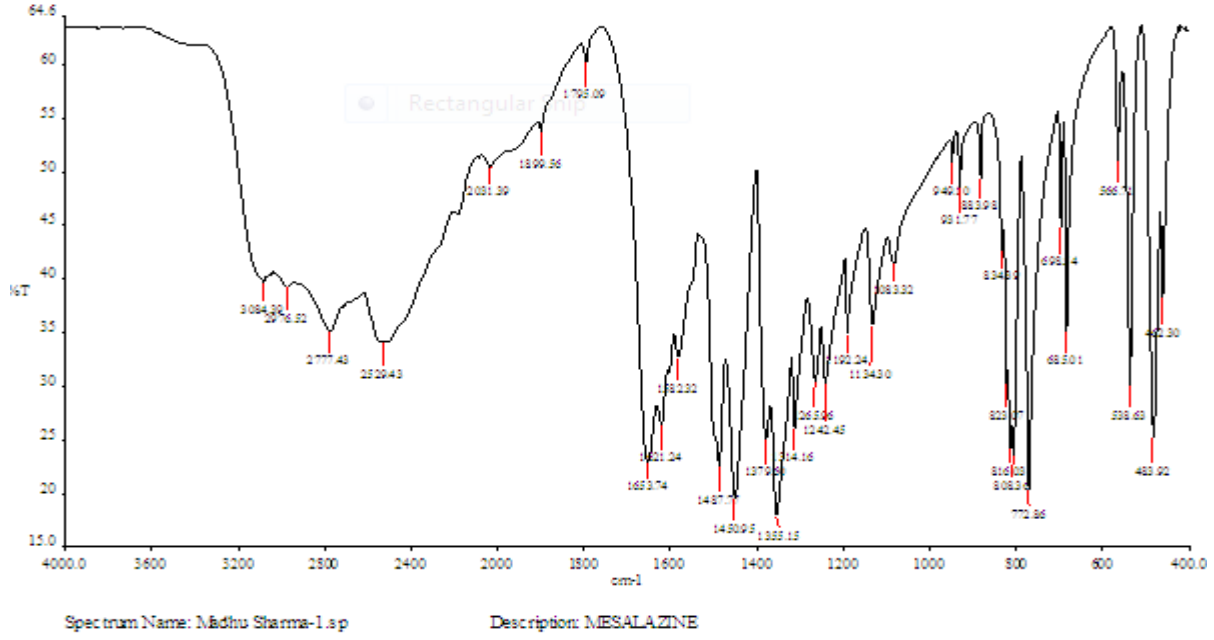


**Figure 1: FTIR spectra of Mesalazine**

**Table 1: Interpretation of FTIR Spectra of Mesalazine:**

| S.No | Functional Group | Peaks Observed |
|---|---|---|
| 1) | C=C stretch of the aromatic group; N-H bond scissoring | 1621.24 |
| 2) | C-H stretch of the aromatic group | 2976.52 |
| 3) | C-C stretching mode | 1487.79 |
| 4) | O-H deformation of the hydroxyl groups | 1582,1487,1450 |
| 5) | C-O stretching mode | 1194.90 |
| 6) | In plane bending mode | 1192.24-1265.96 |
| 7) | C-H bond out of plane bending mode; Ring deformation of the aromatic group | 685.01 |

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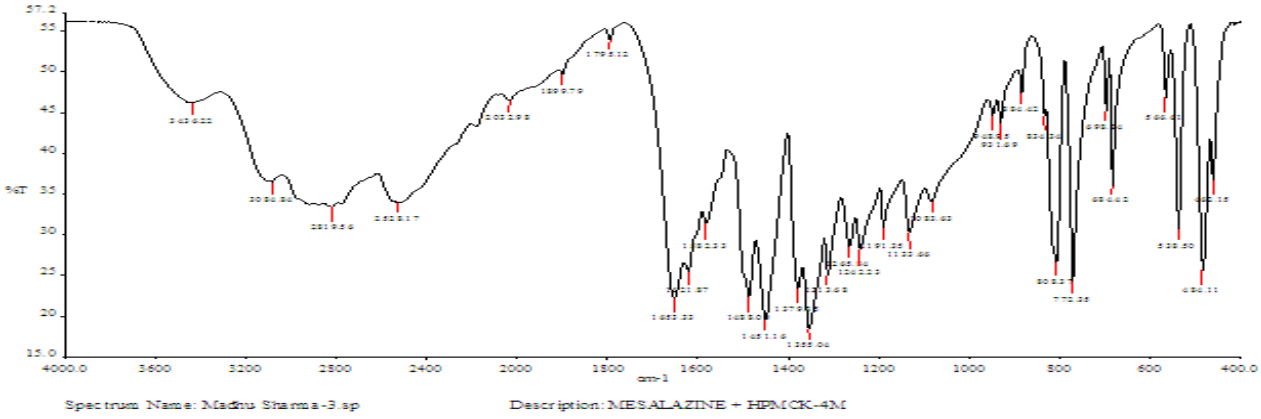


**Figure 2: FTIR Spectra of Mesalazine + HPMC K-4M**

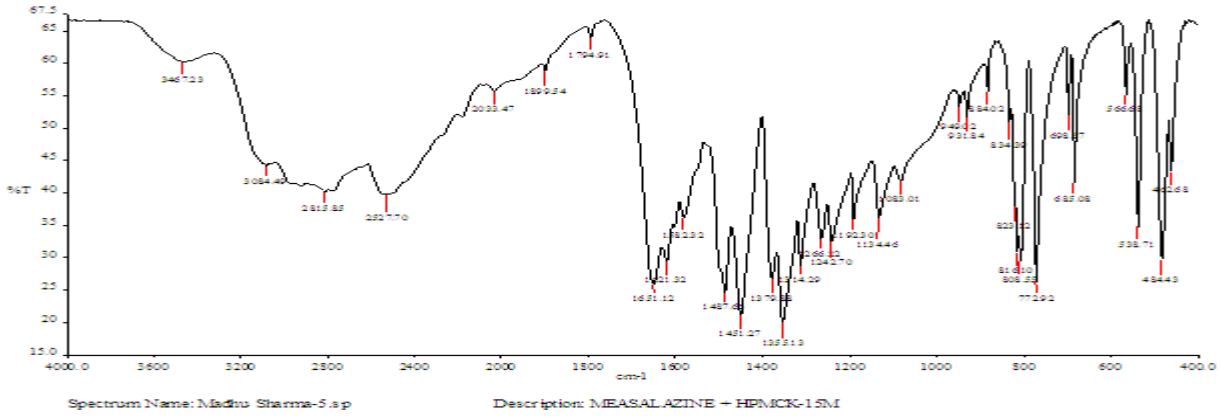


**Figure 3: FTIR Spectra of Mesalazine + HPMC K-15M**

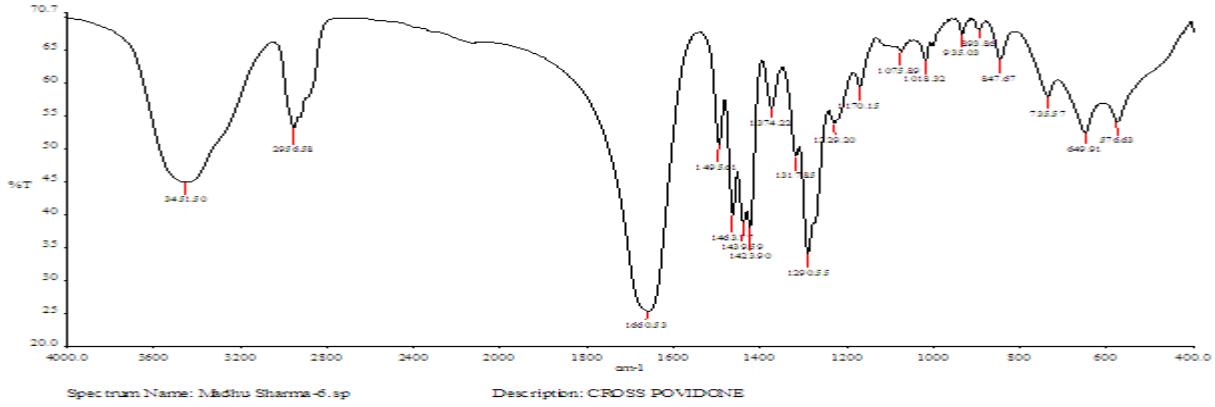


**Figure 4: FTIR Spectra of Mesalazine + Crosspovidone**

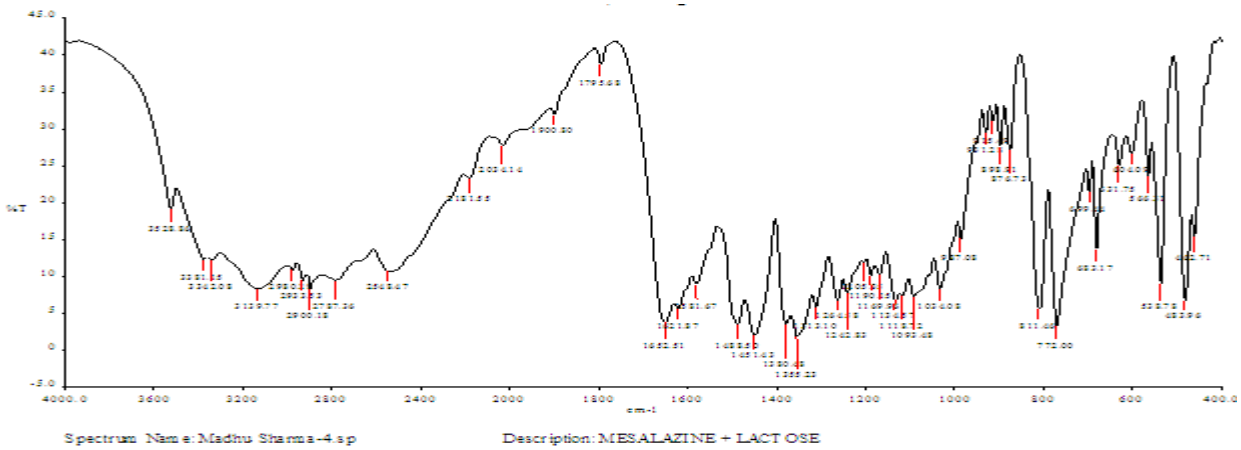


**Figure 5: FTIR Spectra of Mesalazine + Lactose**

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### 3) PREPARATION OF CORE TABLETS

All the ingredients were weighed separately. Mesalazine, lactose, crosspovidone and HPMC (K-4M, K-15M either alone or in combination) were passed through the 16 # sieve and thoroughly mixed and then granulated using starch solution (1% in iso propyl alcohol) as a binder. The granules so obtained were dried at 50-60 °C for 2 hr in the oven. These granules were lubricated with flow promoters like magnesium stearate. The flow properties of the granules were determined. The lubricated granules were compressed into tablets (each 600 mg) using using 12mm concave-faced punch of 10 station Rimek compression machine. Weight variation, hardness, friability, and disintegration test were performed for the core tablets.

**Table 2: Composition of Different Core Tablets of Mesalazine**

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| **Mesalazine** | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 |
| **HPMC K4M** | 120 | 120 | 110 | 110 | 10 | 10 | 60 | 60 |
| **HPMC K 15M** | - | - | 10 | 10 | 110 | 110 | 60 | 60 |
| **Magnesium stearate** | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| **Starch (1%)** | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| **Crosspovidone** | 7.2 (1.2%) | 14.4 (2.4%) | 7.2 (1.2%) | 14.4 (2.4%) | 7.2 (1.2%) | 14.4 (2.4%) | 7.2 (1.2%) | 14.4 (2.4%) |
| **Lactose** | 70.8 | 63.6 | 70.8 | 63.6 | 70.8 | 63.6 | 70.8 | 63.6 |

* ***All quantities are expressed in milligrams***

### 4) EVALUATION OF CORE TABLETS

#### 4.1. Precompressional studies

#### 4.1.1. Flow Properties of Granules

#### 4.1.1.1. Apparent Bulk density:[17]

Apparent bulk density was determined by placing pre-sieved granules into a graduated cylinder and measuring the volume and weight as it is. It was calculated by using formula

**Bulk density = Mass / volume**

#### 4.1.1.2. Tapped density:[17]

Weighed sample of granules was transferred to a graduated cylinder and was tapped for a fixed number of taps (100). Tapped density was calculated by formula given in equation

**Tapped Density = Weight of granules / Tapped volume**

#### 4.1.1.3. Hausner's Ratio: [18]

The Hausner's ratio is a number that is correlated to the flowability of a powder or granular material. It is calculated by formula given in equation

**Hausner's Ratio = Tapped Density / Bulk Density**

#### 4.1.1.4. Compressibility Index: [18]

It is a simple test to evaluate bulk density and tapped density of granules and the rate at which it is packed down. The formula for Carr's index was given in equation

**Carr's Index (%) = [(Tapped density-Bulk Density) x100]/Tapped Density**

#### 4.1.1.5. Angle of Repose:[19]

The angle of repose of blend was determined by the fixed funnel method. The accurately weighed granules were taken in the funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the blend. The blend was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the formula given in equation

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

Where, h and r are the height and radius of the powder cone

#### 4.2. Post Compressional Studies

#### 4.2.1. Shape and Appearance: [20]

Tablets were examined under a lens for the shape of the tablet, and colour was observed by keeping the tablets in light.

#### 4.2.2. Hardness: [21]

Monsanto hardness tester was used for the determination of the hardness. The tablet to be tested was held between a fixed and a moving jaw and reading of the indicator was adjusted to zero. The force applied to the edge of the tablet was gradually increased by moving the screw knob forward .until the tablet broke. The reading was noted from the scale which indicates the pressure required in kg or lb to break tablets.

#### 4.2.3. Thickness:[20]

The crown-to-crown thickness of ten tablets from each batch was determined using vernier caliper. The thickness variation limits allowed are ± 5% of the size of the tablet.

#### 4.2.4. Weight Variation: [20]

Weight variation study was carried out as per USP. Twenty tablets were randomly selected from each batch weighed individually. The average weight and standard deviation was calculated.

#### 4.2.5. Friability:[22,23]

Roche friabilator (Electrolab Mumbai) was used for testing the friability of prepared tablets. Twenty tablets were weighed accurately and placed in the friabilator and rotated at 25 rpm for a period of 4 min. Tablets were dedusted using soft muslin cloth and weighed again. Percentage weight loss was determined by using following formula.

**% Friability = [(Initial weight- Final weight)/ Initial weight] X 100**

**4.2.6. Uniformity of Drug Content:**[24]

For determination of drug content, five tablets from each formulation were triturated using mortar and pestle. An accurately weighed powder equivalent to 400 mg of drug was taken in 100 ml volumetric flask and diluted with sufficient amount of phosphate buffer of pH 6.8 up to mark. Then the sample was sonicated for 1 hr and filtered. An aliquot of the filtrate was diluted suitably and analyzed spectrophotometrically at 331.60 nm against blank. The test was done in triplicate and average drug content was estimated.

**5) PREPARATION OF COATING SOLUTION**

**Table 3: Trials of Coating Using Combination of Eudragit S-100 and L-100**

| Ingredient | Function | F4 | F5 | F6 |
|---|---|---|---|---|
| **Eudragit S-100** | Enteric Polymer | 16 | 16 | 16 |
| **Eudragit L-100** | Enteric Polymer | 16 | 16 | 16 |
| **Diethyl phthalate** | Plasticizer | 4 | 4 | 4 |
| **Acetone** | Solvent | 250 | 250 | 250 |
| **IPA** | Solvent | 250 | 250 | 250 |
| **% coating** | | 8% | 10% | 12% |

***Quantity in grams***

**Parameter Value**

Inlet Air Temperature = 40-45$^0$C

Exhaust Temperature = 30-35$^0$C

Bed Temperature = 38$^0$C

Atomization (bar) = 2

Spray rate (gm/min) = 10

Pan RPM = 10

**6) EVALUATION OF ENTERIC COATED TABLETS**

**6.1. Hardness Test:**[21]

The hardness of the coated tablets was measured using same procedure as described earlier with the help of Monsanto hardness tester. The hardness of various formulations was shown in table 9.

**6.2. Weight Variation Test:**[20]

The weight variation test was carried out for the coated tablets using the same procedure as described earlier and the results were reported in the table 9.

**6.3. *In-vitro* Disintegration Test of Coated Tablets:**[22]

Tablet disintegration was carried by placing one tablet in each tube of the basket and top portion of the each tube was closed with disc. Tablets were firstly tested in 0.1N HCl for 2 h (simulated gastric transit time) to see the damage to the coat. Afterwards, tablets were tested in the phosphate buffer pH 6.8 (simulated colonic pH) till the coating dissolved. Temperature in each case was kept at 37±0.5°C. Disintegration time was reported in min. The time taken for complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured. The experiment was carried out in triplicate.

**6.4. Lag Time Profile:**[25]

Time dependent systems are formulated to undergo a lag time of predetermined span of time of no release, followed by a rapid and complete release of loaded drug. Lag time is the time required to transit from the mouth to the colon.

**6.5. *In-vitro* Dissolution Profile of Mesalazine Coated Tablets:**

*In vitro* drug release studies for the prepared tablets were conducted for a period of 12 hours using USP type-II (Paddle) dissolution apparatus (Electro lab, Mumbai.) at 37±0.5$^o$C and 75 rpm speed using pH 1.2 buffer for initial 2 h, phosphate buffer of pH 7.4 up to 3 h as and phosphate buffer of pH 6.8 for 7 h as dissolution medium. At predetermined interval of time, 10 ml of sample was withdrawn from the dissolution medium and replaced with fresh medium to maintain the volume constant. After filtration and appropriate dilution, the sample solutions were analyzed at 302 nm (acidic media) and 331.70nm (basic media) for Mesalazine by a UV-visible spectrophotometer. The amount of drug present in the samples was calculated and the results were reported in tables 10(a) and 10(b).

**7) COMPARISON OF THE BEST FORMULATION WITH THE MARKETED FORMULATION**

The best formulation was selected on the basis of the release profile and lag time and compared with the marketed formulation (Asacol).

**8) SIMILARITY FACTOR** [26-28]

The similarity factor ($f_2$) is a logarithmic transformation of the sum-squared error of differences between the test $T_t$ and reference products $R_t$ over all time points. It represents closeness of two comparative formulations. Generally similarity factor in the range of 50-100 is acceptable according to US FDA. **Equation for calculation of similarity factor**:

$$f_2 = 50 \text{ X } \log \{[1+ (1/n) \sum_{t=1} * n (R_t-T_t)^2]^{-0.5} *100\}$$

$R_t$ and $T_t$ are the cumulative percentage dissolved at each of the selected n time points of the reference and test product respectively.

**9) STATISTICAL ANALYSIS**

The data was analyzed by using one way Analysis of Variance (ANOVA) followed by Tukey and Dunnett tests by using Graph pad prism software. The value of $p<0.05$ was considered to be statistical significant.

**10) RELEASE KINETIC OF SELECTED FORMULATION** [29-31]

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing.

- Zero order (cumulative % drug release v/s. time).
- First order (log cumulative % drug remaining v/s. time).
- Higuchi model (cumulative % drug release v/s. Square root of time).
- Korsemeyer Peppas model (log cumulative % drug release v/s. log time).

**11) STABILITY STUDY**

The selected batch (F2) was kept at 40°C with 75% RH and the samples were withdrawn at 30, 60 and 90 days for physical and *in-vitro* evaluation of drug release.

**Table 4: Stability Study**

| Parameter | Initial | 1 month (40°C/75%RH) | 2 month (40°C/75%RH) | 3 month (40°C/75%RH) |
|---|---|---|---|---|
| **Description** | Yellowish brown, round shaped | Same | Same | Same |
| **Average weight (mg)** | 681.10 | 681.10 | 681.10 | 681.10 |
| **Hardness (kg/cm$^2$)** | 6.61 | 6.61 | 6.61 | 6.61 |
| | | **Dissolution Study** | | |
| **0** | 0 | 0 | 0 | 0 |
| **1** | 0 | 0 | 0 | 0 |
| **2** | 0 | 0 | 0 | 0 |
| **3** | 0 | 0 | 0 | 0 |
| **4** | 8.52 | 7.99 | 7.85 | 6.90 |
| **5** | 18.45 | 17.85 | 16.72 | 16.49 |
| **6** | 40.56 | 39.71 | 38.89 | 35.66 |
| **7** | 50.42 | 52.02 | 52.11 | 47.02 |
| **8** | 61.05 | 60.28 | 60.25 | 59.77 |
| **9** | 69.58 | 68.66 | 67.41 | 68.98 |
| **10** | 79.23 | 78.59 | 77.90 | 78.86 |
| **11** | 89.42 | 89.42 | 89.45 | 89.31 |
| **12** | 96.53 | 95.49 | 95.46 | 95.90 |

## 6) RESULTS AND DISCUSSION

### 6.1. ANALYTICAL METHOD VALIDATION

**Table 5: Characteristics and Validation Parameters of Mesalazine**

| Validation Parameter | Values | | |
|---|---|---|---|
| | **In 0.1N HCl** | **In PB pH 6.8** | **In PB pH 7.4** |
| **$\lambda_{max}$ (nm)** | 302 | 331.60 | 331.70 |
| **Linearity equation** | Y= 0.046x+0.021 | Y= 0.086x+0.038 | Y= 0.085x+0.018 |
| **Range (µg/ml)** | 1-10 | 1-10 | 1-10 |
| **Intercept (c)** | 0.021 | 0.038 | 0.018 |
| **$R^2$ value** | 0.993 | 0.995 | 0.996 |
| **LOD (µg/ml)** | 0.220 | 0.216 | 0.218 |
| **LOQ (µg/ml)** | 0.685 | 0.673 | 0.688 |

***The low values of LOD and LOQ indicated that the method was sensitive and validated.***

### 6.2. EVALUATION OF CORE TABLETS

#### 6.2.1. Precompressional Studies

##### 6.2.1.1. Flow Properties of Granules

**Table 6: Micromeritic properties of granules**

| Formulation code | Bulk density (gm/ml) | Tapped density(g/ml) | Carr's index (%) | Hausner's ratio | Angle of repose (Θ) |
|---|---|---|---|---|---|
| **F1** | 0.366 | 0.497 | 26.35 | 1.355 | 38.2 |
| **F2** | 0.364 | 0.494 | 26.31 | 1.357 | 37.5 |
| **F3** | 0.361 | 0.490 | 26.33 | 1.357 | 38.6 |
| **F4** | 0.367 | 0.499 | 26.45 | 1.359 | 35.0 |
| **F5** | 0.362 | 0.494 | 26.72 | 1.364 | 32.9 |
| **F6** | 0.367 | 0.491 | 25.25 | 1.337 | 33.4 |
| **F7** | 0.365 | 0.496 | 26.41 | 1.358 | 34.6 |
| **F8** | 0.366 | 0.492 | 25.60 | 1.344 | 37.4 |

***All values are expressed as mean ± SD. n=3.***

From the results of carr's index and hausner's ratio, it was concluded that except formulations F6 and F8, all the formulations possess poor flowability of granules. Formulations F6 and F8 were having passable flow of granules. From the results of angle of repose, it was concluded that except granules of F5 and F6, all other formulations possess poor flow. F5 and F6 were having passable flow.

### 6.2. Post Compressional Studies:

#### 6.2.1. Shape and Appearance

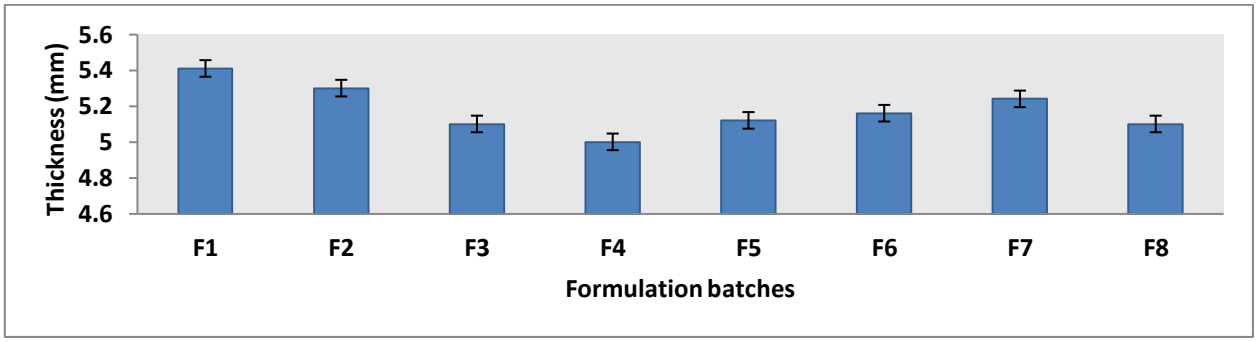
Tablets showed standard concave surfaces with circular shape. Tablets were light brownish in color.

#### 6.2.2. Physicochemical Evaluation of Core Tablets

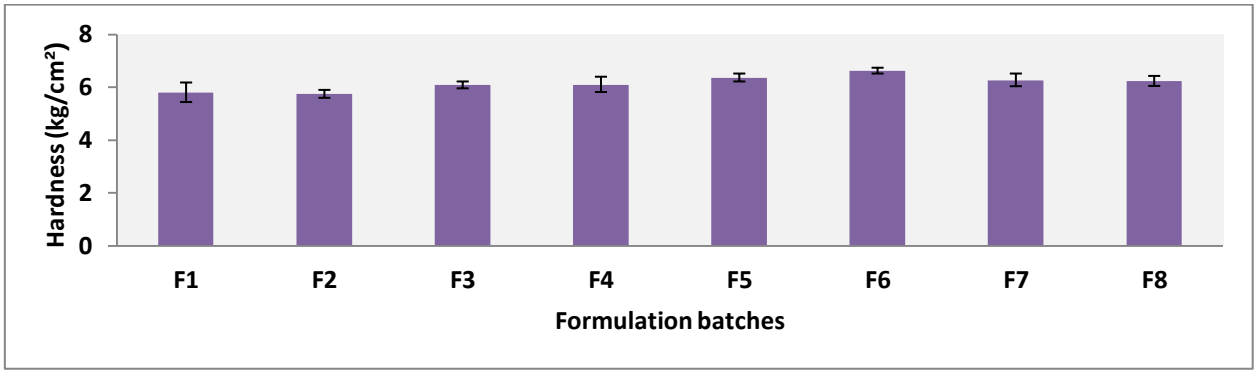
**Table 7: Physicochemical Evaluation of Core Tablets of Mesalazine**

| Formulation Code | Thickness* (mm) | Weight*** (uncoated tablets) (mg) | Hardness* (uncoated tablets) ($kg/cm^2$) | Content uniformity* (%) | Friability** (%) |
|---|---|---|---|---|---|
| **F1** | 5.41±0.771 | 600.20±1.64 | 5.8±0.37 | 99.97±0.556 | 0.17 |
| **F2** | 5.30±0.771 | 600.25±1.48 | 5.74±0.15 | 100.15±0.56 | 0.15 |
| **F3** | 5.10±0.773 | 600.15±1.56 | 6.08±0.13 | 100.18±0.74 | 0.20 |
| **F4** | 5.00±0.771 | 600.10±1.29 | 6.1±0.29 | 99.02±0.950 | 0.21 |
| **F5** | 5.12±0.774 | 600.05±1.3 | 6.36±0.15 | 101.04±0.68 | 0.12 |
| **F6** | 5.16±0.772 | 599.05±1.19 | 6.62±0.11 | 99.57±0.31 | 0.14 |
| **F7** | 5.24±0.772 | 600.25±1.4 | 6.27±0.24 | 95.25±0.25 | 0.17 |
| **F8** | 5.10±0.771 | 600.10±1.29 | 6.23±0.19 | 96.75±0.95 | 0.16 |

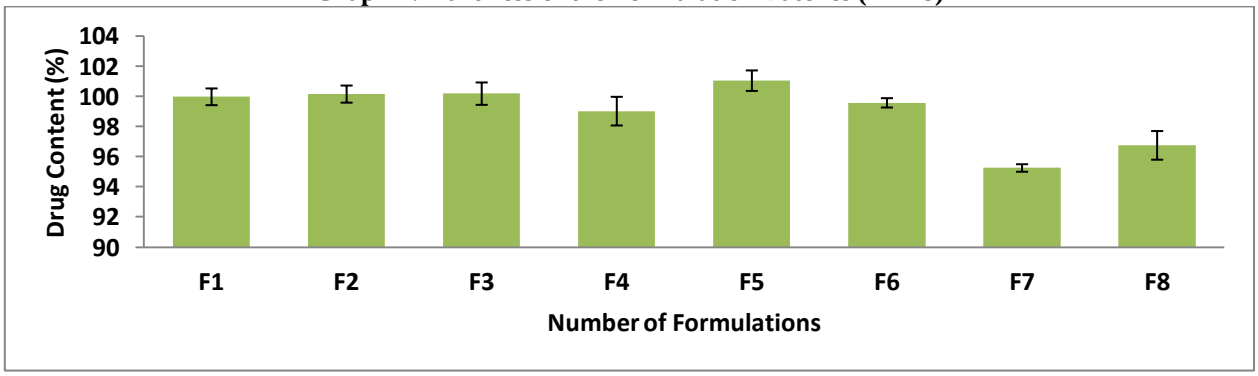
**All values are expressed as mean ± SD, *n=3, ***n=20, ** n=6.*




**Graph 1: Thickness of the Formulation Batches (F1-F8)**

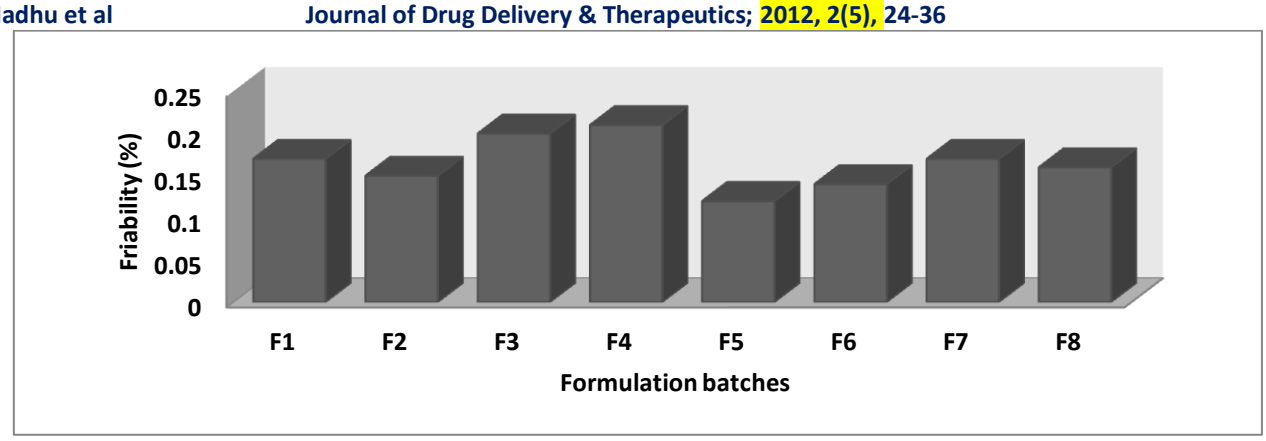



**Graph 2: Hardness of the Formulation Batches (F1-F8)**




**Graph 3: Percent Drug Content of the Formulation Batches (F1-F8)**




**Graph 4: Friability of the Formulation Batches (F1-F8)**

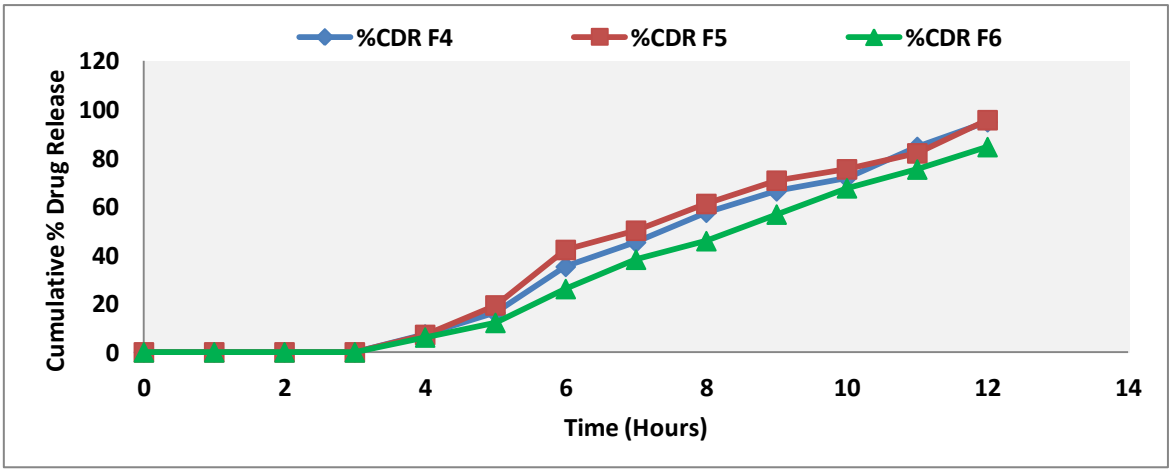
### 6.3. TRIALS OF COATING WITH COMBINATION OF EUDRAGIT L-100 AND EUDRAGIT S-100 IN COMBINATION:

The cumulative *in-vitro* release study was carried out using USP dissolution apparatus type II. Cumulative % drug release after 7 hrs was found to be 45.28%, 50.11% and 38.17% for formulation F4, F5 and F6 respectively. The release before completion of lag time was found to be 16.25%, 19.33% and 12.14% for formulation F1, F2 and F3 respectively.

The results obtained in the *in-vitro* drug release study are tabulated in Table 8. The cumulative percentage of Mesalazine released as a function of time for all the formulations are shown in graph 5. Coating of tablets with Eudragit L-100: Eudragit S-100 in combination showed the lag time of nearly 5 hrs before burst effect. From the result, concluded that the combination of Eudragit L-100: Eudragit S-100 can be successfully utilized to create desired release profile similar to the targeted release profile in future study.

**Table 8: *In-vitro* Release Profile During Trials of Coating With Eudragit S-100 and L- 100 in Combination:**

| Dissolution media | Time (hours) | Cumulative % drug release | | |
|---|---|---|---|---|
| | | F4 | F5 | F6 |
| | 0 | 0 | 0 | 0 |
| 0.1 N HCl | 1 | 0 | 0 | 0 |
| | 2 | 0 | 0 | 0 |
| | 3 | 0 | 0 | 0 |
| PB pH 7.4 | 4 | 7.36 | 7.19 | 6.13 |
| | 5 | 16.25 | 15.33 | 12.14 |
| | 6 | 35.23 | 42.19 | 26.09 |
| | 7 | 45.28 | 50.11 | 38.17 |
| | 8 | 57.54 | 61.14 | 45.82 |
| PB pH 6.8 | 9 | 66.35 | 70.72 | 56.77 |
| | 10 | 71.86 | 75.4 | 67.59 |
| | 11 | 84.63 | 81.97 | 75.32 |
| | 12 | 94.82 | 95.66 | 84.57 |




**Graph 5: *In-vitro* Release Profile of Formulations (F4-F6) During Trials of Coating with Eudragit S- 100 and L- 100 in Combination**

From the results, we have seen that 10% enteric coating gave us more appropriate results as the release of drug at pH 7.4 was less and the drug release at pH 6.8 was more, i.e the drug release was more in the colonic region. While using the 8% enteric coating, more drug was degraded in the small intestine. Also, using the 12% coating, the release of drug in the pH 6.8 (colonic pH) was very less as compared to the 8% and 10% enteric coating. So, the optimized formula of coating consisted of 10% coating of tablets.

### 6.4. EVALUATION OF ENTERIC COATED TABLETS

#### 6.4.1. In- Process Quality Control Tests of Enteric Coated Tablets

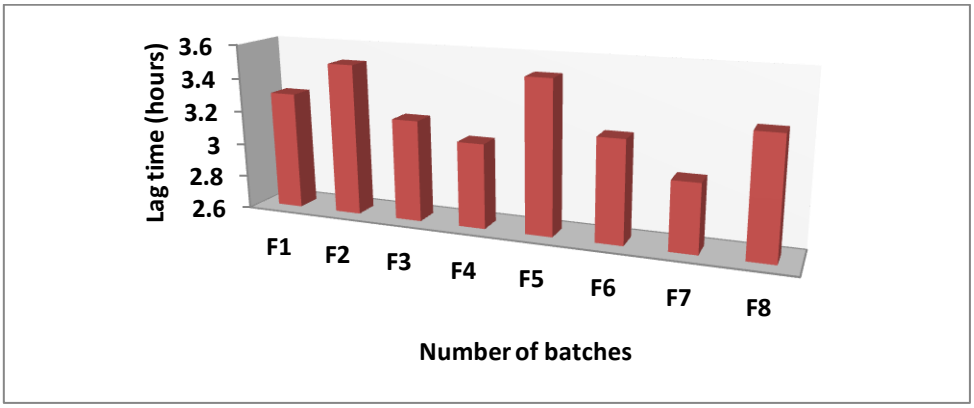
**Table 9: In Process Quality Control Tests Conducted After Coating of Tablets**

| Formulation code | Hardness* (kg/cm²) | Disintegration time** (min) | Weight***(coated tablets) (mg) |
|---|---|---|---|
| **F1** | 6.4±0.30 | 225.89±1.61 | 679.33±2.35 |
| **F2** | 6.61±0.17 | 218.65±1.98 | 681.10±2.66 |
| **F3** | 7.13±0.21 | 223.69±1.98 | 680.19±2.15 |
| **F4** | 7.15±0.34 | 219.76±3.50 | 679.76±2.91 |
| **F5** | 7.25±0.29 | 231.18±2.87 | 679.71±2.93 |
| **F6** | 7.36±0.41 | 230.82±2.25 | 680.38±3.84 |
| **F7** | 7.51±0.53 | 235.45±1.18 | 681.10±2.6 |
| **F8** | 7.42±0.82 | 235.64±2.54 | 685.40±1.75 |

***All values are expressed as mean ± SD, *n=3, **n=6, ***n = 20.***

#### 6.4.2. Lag Time Profile

The lag time profile versus time graph is plotted in graph 6. From the results, it was concluded that F2 and F5 were having sufficient lag time of 3.5 hours. The greater the lag time, more will be the time taken by the dosage form to release the drug.




**Graph 6: Lag Time Versus Number of Batches**

#### 6.4.3. *In-Vitro* Dissolution Profile of Coated Tablets Using Optimized Formula of Coating

The *in-vitro* release study was carried out using USP dissolution apparatus type II. The results obtained in the *in-vitro* drug release study are tabulated in table 10(a) and table 10(b). The cumulative percentage release of Mesalazine as a function of time for all the formulations is shown in graph 7.

**Table 10 (a): *In-vitro* Drug Release Study of Mesalazine Coated Tablets (F1-F4)**

| Dissolution media | Time (hours) | Cumulative % drug release | | | |
|---|---|---|---|---|---|
| | | **F1** | **F2** | **F3** | **F4** |
| | 0 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| **0.1 N HCl** | 1 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 2 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 3 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| **PB pH 7.4** | 4 | 6.74±0.577 | 8.52±2.901 | 6.45±0.991 | 7.86±0.831 |
| | 5 | 17.21±1.352 | 18.45±2.882 | 16.59±1.034 | 17.59±0.621 |
| | 6 | 33.45±1.212 | 40.56±2.967 | 30.22±1.905 | 31.94±0.953 |
| | 7 | 45.45±2.438 | 50.42±2.901 | 40.55±2.734 | 41.66±2.11 |
| | 8 | 56.31±2.516 | 61.05±2.942 | 48.50±2.893 | 50.59±1.89 |
| **PB pH 6.8** | 9 | 65.41±1.243 | 69.58±2.79 | 56.37±2.935 | 59.17±2.99 |
| | 10 | 72.90±2.155 | 79.23±2.91 | 65.28±2.962 | 70.31±2.345 |
| | 11 | 82.19±1.501 | 89.42±2.83 | 73.01±2.994 | 80.82±2.575 |
| | 12 | 91.25±2.347 | 96.53±2.84 | 82.54±2.982 | 90.25±2.341 |

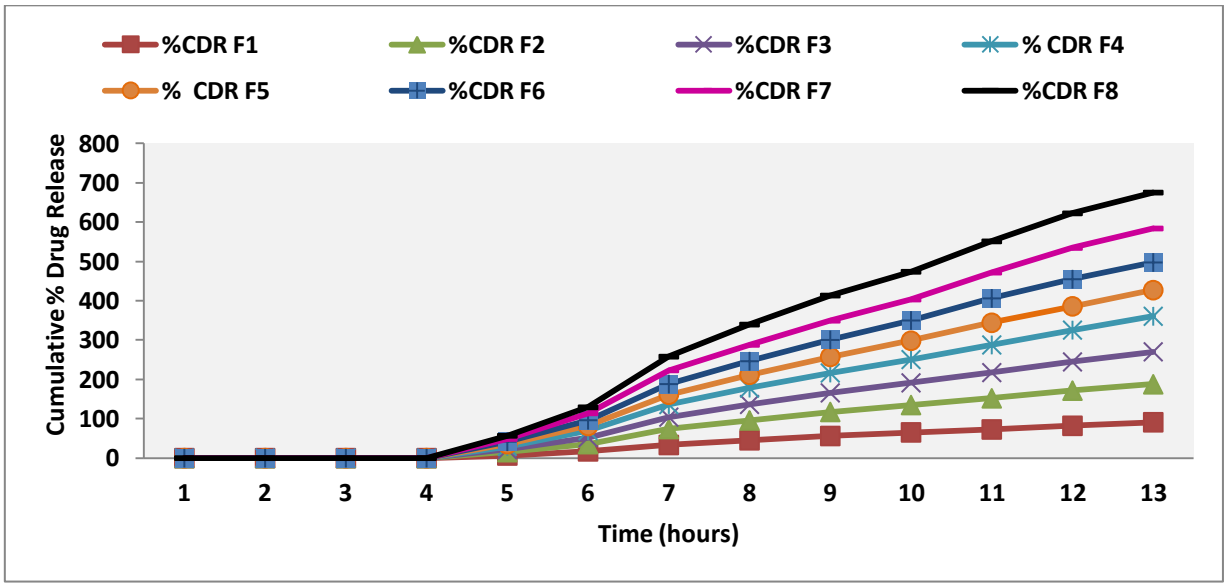
***All values are expressed as mean± S.D***

**Table 10(b): *In-vitro* Drug Release Study of Mesalazine Coated Tablets (F5-F8)**

| Dissolution media | Time (hrs) | Cumulative % drug release | | | |
|---|---|---|---|---|---|
| | | F5 | F6 | F7 | F8 |
| 0.1 N HCl | 0 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 1 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 2 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| | 3 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| PB pH 7.4 | 4 | 6.01±2.212 | 6.12±1.665 | 7.04±0.73 | 8.12±2.89 |
| | 5 | 12.05±0.65 | 14.47±1.36 | 17.59±1.034 | 15.23±2.91 |
| | 6 | 25.18±1.34 | 26.91±1.83 | 34.85±1.902 | 34.86±2.971 |
| | 7 | 33.91±1.28 | 34.40±2.10 | 41.62±1.689 | 51.49±2.995 |
| | 8 | 40.89±2.30 | 43.17±2.81 | 48.92±2.986 | 63.81±2.982 |
| PB pH 6.8 | 9 | 47.81±2.76 | 51.55±2.58 | 53.28±1.901 | 70.56±2.884 |
| | 10 | 56.53±2.94 | 62.09±2.73 | 65.47±2.999 | 79.38±2.978 |
| | 11 | 60.44±2.95 | 68.91±2.47 | 79.58±1.791 | 87.78±2.895 |
| | 12 | 66.40±2.85 | 70.44±2.78 | 86.34±1.902 | 91.27±2.794 |

***All values are expressed as Mean±S.D***




**Graph 7: Effect of Polymer Level on *In-vitro* Release of Mesalazine From Enteric Coated Tablets (F1-F8)**

The results obtained were in agreement with the fact that formulations having higher percentage of HPMC K15M as a matrix former show much more retardation of drug release as compared to the formulations having lower percentage of HPMC K15M. Formulation F5 and F6 show least amount of drug release in dissolution study illuminating the effect of HPMC K15M concentration in the formulations.

***Reason:*** Presence of HPMC K15M forms a much more viscous layer around the tablet allowing less seepage of fluid into the tablet to prolong the drug release. Higher concentration of HPMC K15M provides gel layer which was more viscous as compared to that formed by lower concentration of HPMC K15M.

Instead of higher concentration of HPMC K15M in the formulations F5 and F6, drug release values were almost nearer to the release values obtained from formulations F3 and F4 which were having lower concentration of HPMC K15M.

***Reason:*** This was due to the higher molecular weight of the polymer. Owing to higher molecular weight, polymer chains were also bulkier in nature requiring more time for their unwinding by solvent molecules leading to delay in instant swelling of the polymer. This delay was responsible for the higher drug release from the formulations having higher concentration of HPMC K15M.

**Effect of Polymer Type and Concentration on Drug Release Behaviour:**

From the results of *in vitro* dissolution studies, it was clear that drug release depends upon the type of polymer and concentration of polymer. Drug release was found to be higher in case of formulations based on HPMC K4M.

***Reason:*** Being more viscous in nature, HPMC K-15M reduces the seepage of dissolution media into tablet core, hence sustain the release of drug. Delay in drug release was also owing to the enormous swelling potential of HPMC K15M which led to increase in diffusion path length. Dissolution results in pH 6.8 medium were also in correlation with above explanation.

**Effect of Superdisintegrant Concentration on Drug Release Behaviour:**

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Formulations having higher concentration of crosspovidone showed greater drug release as compared to that having lower concentration in the matrix of tablets. This was also clear from the comparison between dissolution results of formulations F1 and F2, F3 and F4, F5 and F6 followed by F7, F8 in dissolution medium of pH 6.8.

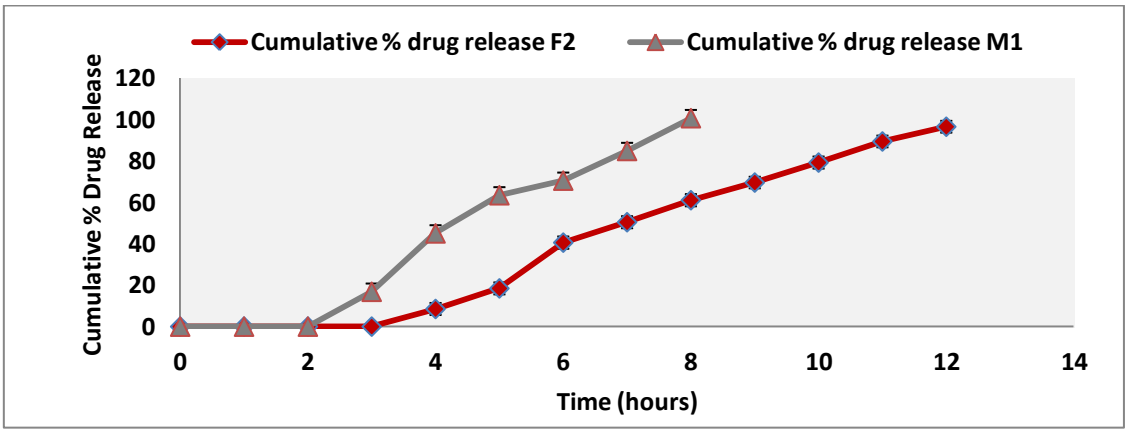
***Reason:*** Increased drug release in formulations containing higher amount of superdisintegrant was due to the formation of pores and cavities in the matrix of tablets. Seepage of the dissolution medium in the matrix of tablets allows rapid swelling of tablet to provide burst effect. Swelling of the tablet depends upon the concentration of superdisintegrant in the formulation; higher amount of superdisintegrant provides higher swelling. But presence of rate controlling hydrophilic polymers do not allows drug to be released at rapid rate and sustained the release of drug. Due to this reason the drug release of formulations F2, F4, F6 and F8 is more than F1, F3, F5 and F7.

## 6.5. COMPARISON OF THE OPTIMIZED FORMULATION WITH THE MARKETED FORMULATION

From the *in-vitro* dissolution studies shown in table 10 (a) and 10(b), it was obvious that formulation F2 achieved more than 95% of the drug release in the time period and it had the lag time of 3.5 hours. So, it was best fitted to be called as optimized formulation. While the release of the marketed formulation was upto 63.449% in the small intestine this is because of the enteric coating using Eudragit S-100 alone. So there was a premature release of drug in small intestine.

Moreover, 100% release of the drug was shown by the marketed formulation in 8 hours. While the formulation F2, being the sustained release formulation released the drug upto 12 hours.

The *in-vitro* release profiles of both the optimized formulation, F2 and the marketed formulation, M1 are plotted in graph 8.




**Graph 8: Comparison of the Marketed Formulation (M1) and the Best Formulation (F2)**

## 6.6. SIMILARITY FACTOR

The result of similarity factor is 20.125. Because the value of similarity factor is less than 50 so, it was concluded that there was a significant difference between dissolution profiles of optimized formulation and the marketed formulation. Because the dissolution profiles are considered to be similar when similarity factor (f2) is between 50 and 100.

## 6.7. STATISTICAL EVALUATION

From ANOVA all the results were expressed as Mean ± Standard Error. The data was analyzed by using one way Analysis of Variance (ANOVA) followed by Tukey and Dunnett tests by using Graph pad prism software. The value of $p<0.05$ was obtained and we conclude that all the values were statistically significant.

**Table 11: Calculation of ANOVA: Single Factor**

| Batch | Analysis of variance | SS | df | MS | F | P-value | $F_{crit}$ |
|---|---|---|---|---|---|---|---|
| **F1** | Between groups | 5937.928 | 1 | 5937.928 | 9.830951 | 0.004489 | 4.259677 |
| **F2** | Between groups | 7303.338 | 1 | 7303.338 | 10.53471 | 0.003438 | 4.259677 |
| **F3** | Between groups | 4485.734 | 1 | 4485.734 | 9.399612 | 0.005304 | 4.259677 |
| **F4** | Between groups | 5328.472 | 1 | 5328.472 | 9.543051 | 0.005016 | 4.259677 |
| **F5** | Between groups | 2825.071 | 1 | 2825.071 | 8.507464 | 0.007556 | 4.259677 |
| **F6** | Between groups | 3462.923 | 1 | 3462.923 | 8.184894 | 0.006679 | 4.259677 |
| **F7** | Between groups | 4893.375 | 1 | 4893.375 | 4.58037 | 0.004944 | 4.259677 |
| **F8** | Between groups | 6930.779 | 1 | 6930.779 | 10.19862 | 0.003901 | 4.259677 |

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### 6.8. KINETICS OF DRUG RELEASE

The value of release exponent (n) was found to be a function of polymer used and the physicochemical property of a drug molecule itself. Kinetic results revealed that, all the formulations followed zero order kinetics as correlation coefficient ($r^2$) values (0.919-0.953) are higher than that of first order release kinetics. The prepared tablets showed supercase-II transport release, as the values of release exponent (n) lies between 2.076-2.213 with correlation coefficient ($r^2$) values upto 0.952, indicating that erosion of polymeric chain was involved in the release process.[31]

**Table 12: Regression Analysis ($R^2$) of Release Data Based on Best Curve-Fitting Method for Different Formulations of Mesalazine Tablets (n=3)**

| Formulation | Zero order | | First Order | | Higuchi | | Korsemeyer Peppas | |
|---|---|---|---|---|---|---|---|---|
| | n | $R^2$ | n | $R^2$ | n | $R^2$ | n | $R^2$ |
| **F1** | 8.646 | 0.950 | -0.080 | 0.851 | 30.29 | 0.781 | 2.213 | 0.924 |
| **F2** | 9.280 | 0.952 | -0.103 | 0.803 | 32.64 | 0.789 | 2.092 | 0.920 |
| **F3** | 7.684 | 0.953 | -0.060 | 0.886 | 26.92 | 0.784 | 2.138 | 0.93 |
| **F4** | 8.318 | 0.952 | -0.074 | 0.833 | 29.05 | 0.778 | 2.091 | 0.952 |
| **F5** | 6.379 | 0.951 | -0.042 | 0.925 | 22.37 | 0.783 | 2.122 | 0.938 |
| **F6** | 6.946 | 0.949 | -0.048 | 0.911 | 24.32 | 0.780 | 2.137 | 0.940 |
| **F7** | 7.942 | 0.950 | -0.066 | 0.850 | 27.81 | 0.781 | 2.076 | 0.923 |
| **F8** | 9.139 | 0.942 | -0.089 | 0.880 | 32.13 | 0.780 | 2.198 | 0.920 |

### 6.9. STABILITY STUDY

The selected formulation (F2) was found to be stable upon storage for 3 months. No change was observed in the appearance, hardness and average weight of the tablet. Also no significant change was observed in the *in-vitro* release of the drug.

## CONCLUSION

From the above results we can conclude that Mesalazine formulations prepared with HPMC K4M, HPMC K 15M and crosspovidone showed acceptable properties like friability, weight variation, hardness etc and *in-vitro* drug release which remained unchanged upon storage for 3 months. However, HPMC K4M, HPMC K15M and crosspovidone (2.4%) based Mesalazine tablets with the formulation code F2 proved to be the formula of choice, since it showed the highest drug release and lag time when compared to the marketed formulation, Asacol. So, Mesalazine tablets can be used in sustained delayed drug delivery in treatment of ulcerative colitis so as to reduce the side effects of drug in stomach and also to reduce the dosing frequency of the drug.

## ACKNOWLEDGEMENT

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