

## Novel Method of Formulation, Optimization and Inviter Evaluation of Eudragit Fs30d Coated Tablets with Biodegradable Polymers for Targeted Colonic Delivery of Azathioprine by Core- In-Cup Tablet Dosage Form.

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**Abstract:** Colonic delivery has gained increasing importance for the delivery of drugs for the treatment of local diseases. To achieve a successful colonic delivery a drug needs to be protected from absorption in the GI tract and abruptly release in to the proximal colon which is considered the optimum site for colon-targeted delivery of drugs. The purpose of this study is to explore the possibility of core in cup tablet as a novel carrier for colonic delivery of a sparingly soluble drug, Azathioprine. The study involves designing a tablet for colon-target which consists of a central blended form of drug and polymer core and were further coated with different concentrations of Eudragit FS30D to thin film and fine thickness and finally the core-in-cup is over coated with pH-sensitive polymer, by spray coat method to provide acid and intestinal resistance. In vitro drug release studies of tablets were carried out in different dissolution media, i.e., 0.1 N HCl (pH 1.2), phosphate buffers pH 6.8 and 7.4, The physicochemical parameters of all the formulations were found to be in compliance with the pharmacopoeial standards. The stability studies of all formulations were performed as per ICH guidelines. The results demonstrated that the tablets coated with Eudragit FS-30D 5%, 7.5 % and 10% w/w showed a sustained release of 98.60% for 24 h in the colon. Drug release study in simulated pH7.4 phosphate buffer (colonic fluid) revealed the delayed release nature of Eudragit coating. Kinetic data proved that the optimized colon drug delivery system was fitted well into first-order model, and apparent lag time was found to be 5 hours, followed by peppas release kinetics. Correlation coefficient ( $r^2$ ) = 0.9999, Values are of mean standard deviation (n=3)  $p < 0.01$  when compared with blank. The enteric coated Eudragit FS30D coated tablets of Azathioprine showed promising site-specific drug delivery in the colon region.

**Keywords:** Azathioprine, Biodegradable gums, Eudragit, film coat, core in cup tablet.

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### I. Introduction

Mainly the colon specific drug delivery system has provided the importance for drugs, which are especially absorbed from colon region by preventing the degradation in upper gastrointestinal tract (GIT). Drug release at this site will ensure maximum therapeutic benefits[1–3] Colon-targeted delivery systems are convenient for treating localized colonic diseases, i.e., Crohn's disease, ulcerative colitis, and constipation, which can be treated most efficiently by local delivery of drugs.[4] The colon-specific delivery system should protect the drug from absorption in the stomach and small intestine, thus prevent a sudden onset of drug release upon entry into less aggressive ambience of the colon. Various drug delivery approaches have been developed for colon-specific drug delivery, which include pH-sensitive system, time-dependent system, pro-drugs, and microflora-activated system to deliver anti-inflammatory agents to the sites of inflammation, and hence systemic drug absorption should be reduced as this leads to unwanted systemic side effects [5–7]. Of all the systems formulated for colon-specific drug delivery, pH-sensitive system and time-dependent system are mostly used.[8–10] The pH variation along the GIT is based on the strategy of using pH as a trigger to achieve drug release in the colon. The high individual variability together with similarity in pH between the small intestine and the colon make the site specificity of pH-dependent system not very reliable[11,12]. Most of the strategies in time-dependent drug delivery are dependent on the principle to delay the drug release until approximate influx in the colon region. Although the relative consistency of transit times in the small intestine is because of the potentially large variation in gastric emptying time, the colon influx time cannot be exactly predicted. Therefore, by suppressing drug release in the stomach and thus reducing the effect of variations in gastric

residence time, appropriate integration of pH-sensitive and time-dependent systems in a single dosage form should improve colon drug delivery.

The release of water-soluble drug from a water-soluble polymeric platform is often rapid, and therefore hydrophobic polymer may be included within the matrix formulation to offer a greater control drug release [13]. Among the various polymers, Eudragit R100 and Eudragit S100 are the hydrophobic polymers which have been used successfully to formulate appropriate sustained release formulations [14]. Core-in-cup tablets have been developed by direct compression based on combination of hydrophobic polymers and a gelling hydrophilic polymer, microcrystalline cellulose, to achieve a 20-h sustained release formulation of Azathioprine tablets using Eudragit

FS30D as coating polymer to produce a delivery system in which the release of drug is modulated. Eudragit coat was employed to delay the penetration of dissolution medium into the matrix, thereby decreasing drug release rate [15].

Azathioprine an immunosuppressive antimetabolite, Thioguanine derivative (AZA) is a prodrug of 6-mercaptopurine that is further metabolized by various enzymes present in the liver and gut have proven efficacy in the treatment of inflammatory bowel disease. Its parent drug 6-mercaptopurine (6-MP), and the closely related 6-thioguanine (6-TG), were originally developed for their anticancer properties, but thiopurines as a class are now more widely used for their anti-inflammatory and immunosuppressant effects. Azathioprine and 6-mercaptopurine may be effective for inducing remission in Crohns disease among patients with chronically active disease. These drugs may reduce the need for steroid treatment and their use may therefore lead to a lower incidence of steroid related side effects

The bioavailability of this drug upon oral ingestion is limited to an extent of 41-50%. It has low biological half life of 3-5 hours. To overcome these drawbacks, the present study was undertaken to investigate the colon targeted drug delivery system of Azathioprine through core and cup technique. Due to the distal location of the colon in the gastrointestinal tract, core and cup technique should prevent drug release in the stomach and small intestine and produce an gradual onset of drug release upon entry into the colon [16].

## **II. Materials and Methods**

**Materials:** Azathioprine was generously gifted by Neo Laboratories Ltd. (Mumbai, India) and RPG Life Sciences Ltd. (Mumbai, India). All the chemicals utilized were of suitable analytical grade and used as and when required. Biodegradable-natural Gums were procured from Nutriroma Company at Hyderabad and all the chemicals required were purchased from national scientifics, Guntur.

### **Experimental design**

**Preparation of Azathioprine core tablet:** The controlled release matrix tablet of Azathioprine was prepared by wet granulation method [17]. The required quantities of Azathioprine, PVPK-30 (as a binder), gum karaya or cashew nut tree gum or gum kondagogu (as a polymers) and lactose (as a diluent) were weighed as per formula given in **Table 1** and these ingredients were mixed uniformly and prepared a wet mass by addition of binder solution. The wet mass was passed through sieve number #12 and allowed to drying for 30 minutes in a tray dryer for 60°C. The dried granules were passed through the sieve number #16 and finally lubricated with talc and magnesium stearate. The obtained dry granules were weighed into individual tablets and finally compressed into the tablet by 16 station rotary tablet compression machine using 9mm flat punches. (RIMEK, Karnavati Engineering Ltd., Gujarat, India).. The core tablets of AZA having an average weight of 300 ± 5 mg were prepared by direct compression. Different gums were used as binders cum hydrophilic matrix former, anhydrous lactose as diluents and magnesium stearate as lubricant, Talc as glidant [18-19]. Fifty core tablets and compression-coated tablets were prepared in duplicate

### **Preparation of cup tablet:**

The cup formulations were formulated by direct compression technique. In which the required quantities of Eudragit L100, Eudragit S100 and microcrystalline cellulose according to the formula shown in **Table- 2**, were weighed and mixed uniformly and finally the powder mixture was compressed by 16 station rotary tablet compression machine by using special punch designed and fabricated, to prepare cup tablets. The newly designed upper 12 mm punch has protrusion and lower punch (12mm) remains flat faced [20].

### **Preparation of aqueous dispersion containing Eudragit FS30D**

To prepare the Eudragit FS30D coating dispersion a 30% (w/w) aqueous Eudragit FS30D dispersion was used. Polysorbate 80 as a wetting agent and glyceryl monostearate as a glidant were added to water and the mixture was heated at 60°C by stirring for 10 min at 50 rpm until a fine homogeneous dispersion was obtained. After cooling this dispersion was gently added to Eudragit FS30D dispersion and mixed by magnetic stirrer. For

this coating dispersion no plasticizer was needed in the formulation since it exhibits a minimum film-forming temperature of 14<sup>0</sup> C and low glass transition temperature [21].

#### **Film coating on core tablets with FS30D spray solution**

Placed the core tablets into a coating pan, the coating solution was sprayed over the tablets by R&D coater, rotating with a speed of 15 rpm, the pressure of the spray gun was maintained at 0.1 M.Pa and the temperature was maintained at 35-40<sup>0</sup>C[22].

**Preparation of core in cup tablet:** The cups were placed in a 12mm die cavity and core tablet was inserted into the cups and compressed with 12mm flat faced punches. The composition of the core in cup tablets was given in **Table 3**.

#### **Evaluation of granules - Precompression parameters**

##### **Angle of repose**

The angle of repose of granules was determined by the funnel method. The accurately weighed granules were taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of the granules. The granules were 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 following equation:

$$\tan \theta = h/r,$$

Where  $\theta$  = angle of repose, h = height, and r = radius.

##### **Bulk density**

Bulk density is defined as ratio of total mass of powder to the bulk volume of powder. Bulk volume is the volume occupied by a certain mass of powder when gently poured into a measuring cylinder. It was measured by pouring initially weighed powder into a measuring cylinder and the volume (bulk volume) was noted. From this, the bulk density was calculated according to the formula mentioned below. It is expressed in g/ml and is given by

$$D = M/V$$

Where M is the mass of powder and V is the bulk volume of the powder.

##### **Tapped density**

It is the ratio of total mass of the powder to the tapped volume of the powder. Volume was measured by tapping the powder for 100 times and then the tapped volume was noted. It is expressed in g/ml and is given by

$$D = M/V$$

where M is the mass of powder and V is the tapped volume of the powder.

##### **Compressibility index**

It indicates powder flow properties. It is expressed in percentage and is given by

$$I = (D_t - D_b) \times 100,$$

where  $D_t$  is the tapped density and  $D_b$  is the bulk density of the powder.

##### **Hausner ratio**

The Hausner ratio is a number that is correlated to the flowability of powder. It is calculated by the following formula:

$$\text{Hausner ratio} = D_t / D_b$$

where  $D_t$  is the tapped density and  $D_b$  is the bulk density.

A Hausner ratio greater than 1.25 is considered to be an indicator of poor flow ability

#### **Drug- Excipient Compatibility Studies by FT-IR: Characterization**

The Fourier transform infrared (FTIR) spectra of pure drug (Azathioprine), Eudragit R100, EudragitS-100, physical mixture of Azathioprine–Eudragit, Gum karaya, kondagogu, cashew nut tree gum were recorded using a FTIR spectrophotometer (Perkin Elmer Spectrum 400, England) according to the KBr disk technique. The smoothing of the spectra and the baseline correlation procedures were applied. The FTIR measurements were performed in the scanning range of 4000-400  $\text{cm}^{-1}$  at ambient temperature. The results of Pre compression parameters were reported in **Table-4**

### **Post compression evaluation of Azathioprine tablets Weight variation**

For estimating weight variation, 20 tablets of each formulation were weighed using an electronic balance (Denver Instrument, Gottingen, Germany) and the test was performed according to the official test.

### **Thickness**

The thickness of the tablet was measured using a Digital Vernier Calliper (Mitutoya Digimatic Calliper, Kanagawa Japan).

### **Hardness**

The crushing strength of ten tablets was measured using Monsanto tablet hardness tester (Interlabs, Ambala, India). A tablet hardness of about 5-7 kg/cm is considered adequate for mechanical stability.

### **Friability**

The friability of the tablets was determined in Roche Friabilator (Model 902, EI product, Panchkula, India). Six tablets were weighed accurately from each batch of tablets and placed in the tumbling chamber and rotated at 25 rpm for a period of 4 min. Tablets were taken and again weighed. The percentage loss was determined by using the formula

$$\% \text{ Friability} = \frac{\text{Initial weight of tablets} - \text{Final weight of tablets}}{\text{Initial weight of tablets}} \times 100$$

The results of Post compression parameters were reported in **Table-5**

### **Enteric coating method**

#### **Over coat of core-in-cup tablets with Eudragit FS 30D**

The coating dispersion of Eudragit FS 30D was spray coated over the core-in-cup tablet. The tablets were coated to a 5%, 7.5 % and 10% w/w total weight gain.

**Coating process:** Placed the core in cup tablets into a coating pan, the coating solution was sprayed over the tablets by R&D coater, rotating with a speed of 15 rpm, the pressure of the spray gun was maintained at 0.1 M.Pa and the air temperature was maintained at 35-40°C. The tablets were coated to a 5%, 7.5 % and 10% w/w total weight gain.

## **III. Methodology**

### **Determination of UV Absorption Maxima, Stock and working standard solution**

Accurately weighed quantity of 50 mg of Azathioprine was transferred into a 100ml volumetric flask. Then added 30 ml of methanol and 1ml of ammonia solution to the flask swirled and sonicated for 5 minutes. Diluted up to the volume with Methanol and mixed well. 10 ml of this solution was transferred to a 50 ml volumetric flask, diluted with water to volume and mixed well. This solution has given a concentration of 100 µg/ml. The resulting solution was scanned in UV range (200nm–400nm). In spectrum Azathioprine showed absorbance maximum at 281 nm.

For the estimation of Azathioprine and Linearity study different aliquots of Azathioprine from the stock solution has to be diluted subsequently with 0.1N HCl to get a series of dilutions containing 1, 2, 4, 6, 8 and 10 µg/ml of solution. The absorbance of these solutions was measured at 281 nm against blank.

### **Stability Study**

The stability study was conducted according to International Conference on Harmonisation (ICH) guidelines. All the formulations were stored in aluminum packaging laminated with polyethylene (cellophane packets) and kept in humidity chamber (Oswal Scientific, Chandigarh, India) at 30°C ± 2°C/65% ±5% RH (room temperature studies) and 40°C ± 2°C/75% ±5% RH (accelerated temperature studies) for 6 months. The tablets were analyzed after 0 day, 1 month, 2 months, 3 months, and 6 months. At the end of the study period, the tablets were observed for the change in physical appearance, color, and drug content.[23]

### **In vitro drug release study**

*In vitro* drug release study was performed as per the method described previously by Bipul Nath., and Lila Kanta Nath[24]. Six compression-coated tablets were placed in 900 mL 0.1 (M) HCl solution (37± 0.50C) of pH 1.2 contained in 6 vessels of USP-II dissolution rate test apparatus (TDP-06P, Electro Lab, Mumbai, India) and rotated with paddles at 100 rpm. The pH of the solution was increased after 2 h to 7.4 by adding 200mL 0.2 (M) trisodium orthophosphate dodecahydrate (simulated colonic fluid). After an additional 3h period, the pH of the solution was changed to 6.8 by adding 5 mL 2 (M) HCl till more than 90% of drug release occur. Eudragit FS30D is a relatively new pH dependent methacrylic acid polymer for colonic delivery purposes. The earlier reported threshold pH values for Eudragit FS30D polymer was 6.8, 7.2 or above and 7.5.

In order to test the suitability of Eudragit FS30D for colon targeting, the pH dissolution profile of the tablets with the 5%, 7.5 % and 10% w/w coating level were investigated in 0.2M phosphate buffers at pH 6.8 and 7.2, Aliquots were removed at predetermined times and replenished immediately after each withdrawal with the same volume of fresh media maintained at 37°C. The aliquots following suitable dilution were analyzed at 281 nm using UV Spectrophotometer [Lab India]. The amount of AZA released from the tablets was calculated using calibration curves drawn in the respective dissolution medium [24]. The results of 24 hr invitro dissolution study was reported in **Table-6** of which we reported only the 10%

## **IV. Results and Discussion**

### **Characterization of Granules**

The granules for AZA polymer coated tablets were prepared by wet granulation method according to the formula shown in **Table 1**. The granules were characterized with respect to angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio. The parameters for evaluation of granules are depicted in **Table 7**. The angle of repose of different formulation batches from F1 to F9 was found to be from  $22^\circ \pm 0.7^\circ$  to  $29^\circ \pm 0.4^\circ$ . The angle of repose was less than  $30^\circ$  for all the formulation batches of granules, indicating good flow behavior. Similarly, bulk density and tap density of all the formulation batches from F1 to F9 were found to be from  $0.37 \pm 0.5$  to  $0.66 \pm 0.8$  g/ml and from  $0.44 \pm 0.3$  to  $0.76 \pm 0.5$  g/ml, depicting good flow properties of the granules. The Carr's index of all formulation batches was in the acceptable range from  $11.9 \pm 0.5$  to  $15.9 \pm 0.3$ . The Hausner ratio of all formulation batches from F1 to F9 was found to be from  $1.13 \pm 0.3$  to  $1.19 \pm 0.6$ . The Hausner ratio less than 1.25 indicates good flowability [25].

### **FTIR Study**

The FTIR spectra of Azathioprine, Eudragit R100, Eudragit S-100, and physical mixture are reported. FTIR spectrum of Azathioprine showed characteristic peaks at  $3561.6 \text{ cm}^{-1}$  due to the nitro group, at  $1676.5 \text{ cm}^{-1}$  due to presence of N–O stretch, the C = C (aromatic stretch) at  $1602.9 \text{ cm}^{-1}$ , –CH<sub>3</sub> bend at  $1457.2 \text{ cm}^{-1}$ , FTIR spectrum of Eudragit S-100 showed the peak at  $2953.9 \text{ cm}^{-1}$  due to presence of O–H (carboxylic acid), at  $1450.7 \text{ cm}^{-1}$  due to –CH<sub>3</sub> bend, and at  $1731.2 \text{ cm}^{-1}$  due to the presence of C = O (ester). FTIR spectrum of Eudragit R100 showed the peak at  $3432.1 \text{ cm}^{-1}$  due to the presence of tertiary amine, at  $1731.4 \text{ cm}^{-1}$  due to the presence of C = O (ester), and at  $1450.2 \text{ cm}^{-1}$  due to –CH bend. FTIR spectrum of the physical mixture of drug and polymer showed the peak at  $1728.6 \text{ cm}^{-1}$  due to the presence of C = O (ester), at  $1174.8 \text{ cm}^{-1}$  due to the presence of C–O stretch, the C = C (aromatic stretch) at  $1604.1 \text{ cm}^{-1}$ , CH<sub>3</sub> bend at  $1453.1 \text{ cm}^{-1}$ , at  $1728.6 \text{ cm}^{-1}$  due to the carboxyl group, and at  $2960.9 \text{ cm}^{-1}$  due to presence of O–H (carboxylic acid). Therefore, FTIR study concluded that no interaction occurred between the drug and polymer.

### **Formulation Aspects of Core Tablets**

The weight of each tablet was determined to be within the range of  $300 \pm 5$  mg in order to maintain the relatively constant volume and surface area. The core tablet (300mg each) was prepared at average tensile strength of  $4.0 \text{ Kg/cm}^2$  and average diameter of 8 mm and thickness 4mm.

### **Evaluation of the Eudragit Coated Tablets.**

The weight variation was in the range of  $645 \pm 2.09$  to  $650 \pm 1.98$  mg and friability was less than 1%. Uniformity in drug content was found among different batches of the tablet, and the drug content was more than 95%.

### **Influence of Coating Formulation variables on Drug Release**

The core tablet was successfully coated by coating technique and further core-in-cup tablet was coated with varying proportion of Eudragit FS30D. The coating compositions of the various formulations are presented in Table 4. The results of the *in vitro* dissolution studies of different batches of coated tablets indicated that increase in concentration of Eudragit FS30D from 5% to 7.5% w/w and 10% w/w and keeping constant weight gain in thickness of polymers at 10% w/w, the lag time (the time required for drug release in SCF) was significantly increased to 5h. The lag time was determined by separately running dissolution studies of Eudragit coated (5% to 7.5% w/w and 10% w/w) tablets in SCF for 5 hours at minimum time intervals. The amount of Eudragit coat was the key factor for such lag time. Lower amount of Eudragit coat shows shorter lag time, and higher amount shows longer lag time. Core-in-cup tablet with a coating level of 10% w/w showed a lag time of 5 hr corresponds to time required to reach colonic region.

### **Effects of Concentration of Eudragit on Drug Release**

To study the effect of concentration of Eudragit FS30D, its concentration in the coating solution was kept at 5, 7.5, 10% w/w for the batch. The result of the *in vitro* release profile from these formulations is shown

in Figures. It is observed that concentration of Eudragit has direct effect on drug release. The formulation F3<sup>3</sup> containing highest concentration (10% w/w) of Eudragit FS30D in the coating composition released more than 90% of AZA after 17h of the dissolution study. This might be due to the reason that an increase in the amount of Eudragit coated Formulation ( $F_{\text{kondagogu}}^{3(10\%)} > F_{\text{karaya}}^{6(10\%)} > F_{\text{cashew}}^{9(10\%)}$ ) it became more susceptible to Colonic pH resulting in longer lag time (5h) for drug release.

### Kinetic analysis of dissolution data

To study the mechanism of drug release from the Eudragit film coated tablets, the release data were fitted to zero-order, first-order, and Higuchi equation. The dissolution data were also fitted to the well-known exponential equation (Korsmeyer–Peppas equation), which is often used to describe the drug release behavior from polymeric systems:  $\log Mt/M = \log k + n \log t$ ,

where  $M$  is the amount of drug released at time  $t$ ,  $M_{\infty}$  is the amount of drug released after infinite time,  $k$  is a release rate constant incorporating structural and geometric characteristics of the tablet, and  $n$  is the diffusional exponent indicative of the mechanism of drug release.

To determine the release exponent for different batches of tablets, the log value of percentage drug dissolved was plotted against log time for each batch. A value of  $n = 0.45$  indicates  $> 0.45$  Fickian (case I) release,  $< 0.89$  indicates non-Fickian (anomalous) release, and  $n > 0.89$  indicates super case II type of release. Case II generally refers to the erosion of the polymeric chain, and anomalous transport (non-Fickian) refers to a combination of both diffusion and erosion controlled drug release [26].

Fig: 1 Calibration curve of Azathioprine in 0.1N HCL.

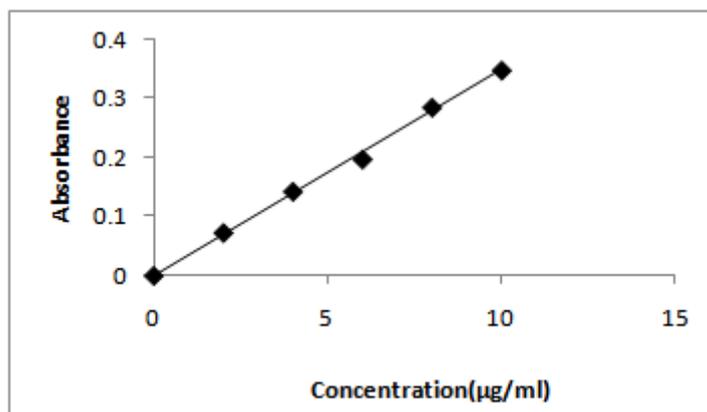


Fig: 2 Calibration curve of Azathioprine in pH 6.8 Phosphate buffer.

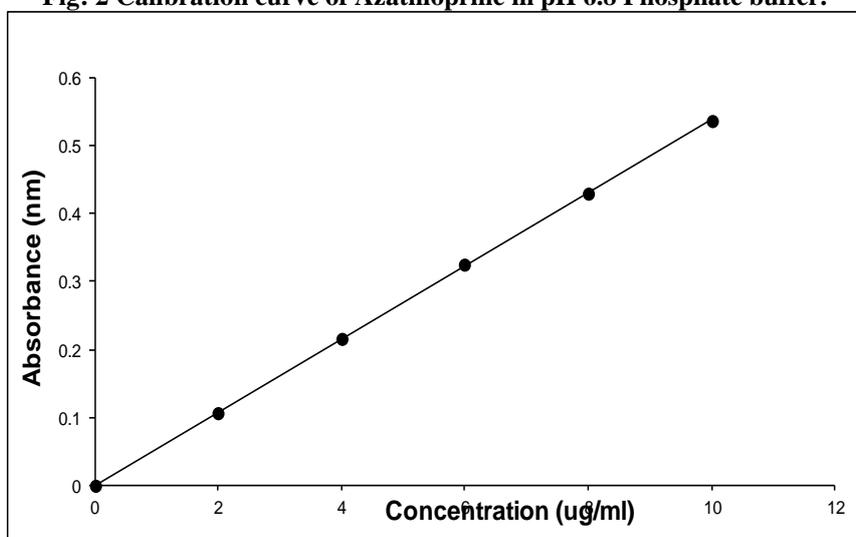


Fig: 3 Calibration curve of Azathioprine in pH 7.4 Phosphate buffer.

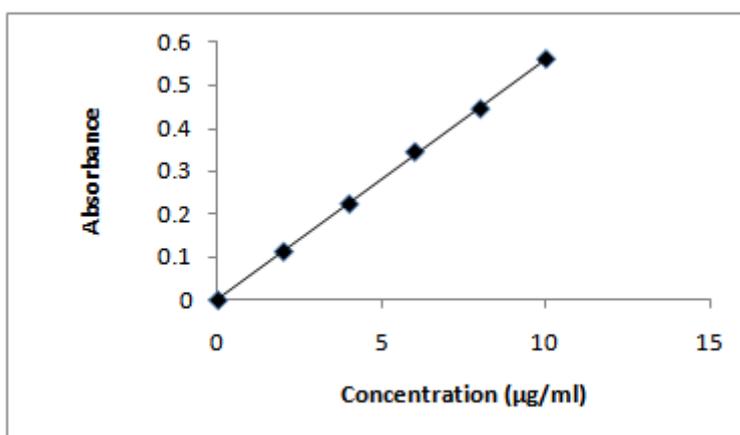


Table1: Azathioprine Core formulations

| Ingredients         | F1 (mg) | F <sub>2</sub> | F <sub>3</sub> | F <sub>4</sub> | F <sub>5</sub> | F <sub>6</sub> | F <sub>7</sub> | F <sub>8</sub> | F <sub>9</sub> |
|---------------------|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Azathioprine        | 50      | 50             | 50             | 50             | 50             | 50             | 50             | 50             | 50             |
| Pvp k30             | 10      | 10             | 10             | 10             | 10             | 10             | 10             | 10             | 10             |
| Gum kondagogu       | 25      | 50             | 75             | -              | -              | -              | -              | -              | -              |
| Gum karaya          | -       | -              | -              | 25             | 50             | 75             | -              | -              | -              |
| Cashew nut tree gum | -       | -              | -              | -              | -              | -              | 25             | 50             | 75             |
| Lactose             | 111     | 86             | 61             | 111            | 86             | 61             | 111            | 86             | 61             |
| Magnesium stearate  | 2       | 2              | 2              | 2              | 2              | 2              | 2              | 2              | 2              |
| Talc                | 2       | 2              | 2              | 2              | 2              | 2              | 2              | 2              | 2              |
| Tablet Total Weight | 200     | 200            | 200            | 200            | 200            | 200            | 200            | 200            | 200 (mg)       |

Table 2: Azathioprine Cup formulations

| Ingredients    | C <sub>1</sub> | C <sub>2</sub> | C <sub>3</sub> | C <sub>4</sub> | C <sub>5</sub> | C <sub>6</sub> | C <sub>7</sub> | C <sub>8</sub> | C <sub>9</sub> |
|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Eudragit R 100 | 200(mg)        | 200            | 200            | 200            | 200            | 200            | 200            | 200            | 200            |
| Eudragit S 100 | 200            | 200            | 200            | 200            | 200            | 200            | 200            | 200            | 200            |
| MCC            | 42             | 42             | 42             | 42             | 42             | 42             | 42             | 42             | 42             |
| Mg. stearate   | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              |
| Talc           | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              |
| Total          | 450            | 450            | 450            | 450            | 450            | 450            | 450            | 450            | 450(mg)        |

Table: 3. Azathioprine Core in cup formulations

| S.No | Core in cup formulations | Combination of core and cup formulations     |
|------|--------------------------|--|
| 1    | CC <sub>1</sub>          | F <sub>1</sub> (core) + C <sub>1</sub> (cup) |
| 2    | CC <sub>2</sub>          | F <sub>1</sub> (core) + C <sub>2</sub> (cup) |
| 3    | CC <sub>3</sub>          | F <sub>1</sub> (core) + C <sub>3</sub> (cup) |
| 4    | CC <sub>4</sub>          | F <sub>1</sub> (core) + C <sub>4</sub> (cup) |
| 5    | CC <sub>5</sub>          | F <sub>1</sub> (core) + C <sub>5</sub> (cup) |
| 6    | CC <sub>6</sub>          | F <sub>1</sub> (core) + C <sub>6</sub> (cup) |
| 7    | CC <sub>7</sub>          | F <sub>1</sub> (core) + C <sub>7</sub> (cup) |
| 8    | CC <sub>8</sub>          | F <sub>1</sub> (core) + C <sub>8</sub> (cup) |
| 9    | CC <sub>9</sub>          | F <sub>1</sub> (core) + C <sub>9</sub> (cup) |

**Table: 4 Evaluation of Pre compression parameters and characterization of granules**

| Parameters         | F1       | F2       | F3       | F4       | F5       | F6       | F7       | F8       | F9       |
|--------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Angle of repose(0) | 27±0.8   | 23±0.5   | 28±0.3   | 25±1.1   | 22±0.7   | 29±0.4   | 24±1.2   | 27±0.2   | 25±0.5   |
| Bulk density g/ml  | 0.55±0.5 | 0.39±0.2 | 0.66±0.8 | 0.48±0.3 | 0.59±0.6 | 0.45±0.2 | 0.59±0.4 | 0.63±0.7 | 0.37±0.5 |
| Tap density g/ml   | 0.63±0.4 | 0.46±0.7 | 0.76±0.5 | 0.55±0.3 | 0.67±0.8 | 0.52±0.2 | 0.68±0.4 | 0.73±0.6 | 0.44±0.3 |
| Carrs index        | 12.7±0.4 | 15.2±0.6 | 13.1±0.2 | 12.7±0.7 | 11.9±0.5 | 15.5±0.8 | 13.2±0.2 | 13.7±2.6 | 15.9±0.3 |
| HaunTERS ratio     | 1.14±0.7 | 1.18±0.2 | 1.15±0.4 | 1.14±0.1 | 1.13±0.3 | 1.15±0.5 | 1.15±0.8 | 1.16±0.2 | 1.29±0.6 |

**Table-5 Post compression parameters of Azathioprine tablets**

**5A) Evaluation Tests of Azathioprine core-in-cup Tablets Formulated with Gum kondagogu in Different Ratios.**

| Formulations   | Hardness (kg/cm <sup>2</sup> ) | Tablet weight variation (mg) | Friability (%) | Drug content (%) |
|----------------|--------------------------------|------------------------------|----------------|------------------|
| F <sub>1</sub> | 4.7±0.021                      | 650±1.7                      | 0.40±0.010     | 100.14±0.13      |
| F <sub>2</sub> | 4.5±0.025                      | 650±1.4                      | 0.34±0.018     | 99.78±0.15       |
| F <sub>3</sub> | 4.8±0.032                      | 650±1.5                      | 0.45±0.024     | 99.56±0.11       |

**5B) Evaluation Test of Azathioprine core-in-cup Tablets Formulated with Gum karaya in Different Ratios.**

| Formulations   | Hardness (kg/cm <sup>2</sup> ) | Tablet weight variation (mg) | Friability (%) | Drug content (%) |
|----------------|--------------------------------|------------------------------|----------------|------------------|
| F <sub>4</sub> | 4.6±0.03                       | 650±1.3                      | 0.61±0.03      | 100.1±0.38       |
| F <sub>5</sub> | 5.1±0.04                       | 650±1.5                      | 0.67±0.04      | 100.7±0.87       |
| F <sub>6</sub> | 5.2 ±0.03                      | 650±1.6                      | 0.69±0.02      | 99.67±0.08       |

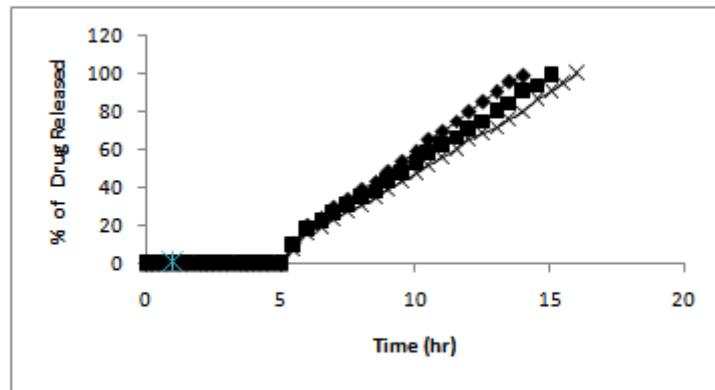
**5C) Evaluation Test of Azathioprine core-in-cup Tablets Formulated with Cashew nut tree gum in Different Ratios.**

| Formulations   | Hardness (kg/cm <sup>2</sup> ) | Tablet weight variation (mg) | Friability (%) | Drug content (%) |
|----------------|--------------------------------|------------------------------|----------------|------------------|
| F <sub>7</sub> | 4.7 ±0.02                      | 650±1.3                      | 0.42±0.02      | 100.34±0.11      |
| F <sub>8</sub> | 4.4 ±0.04                      | 650±1.5                      | 0.39±0.05      | 99.89±0.14       |
| F <sub>9</sub> | 4.8 ±0.02                      | 650±1.7                      | 0.40±0.03      | 100.21±0.16      |

**Table-6 In-vitro Dissolution data consisting of Azathioprine core in cup tablets prepared with Gum kondagogu, Gum karaya and cashew nut tree gum in different ratios with Eudragit FS30D coated 10% w/w**

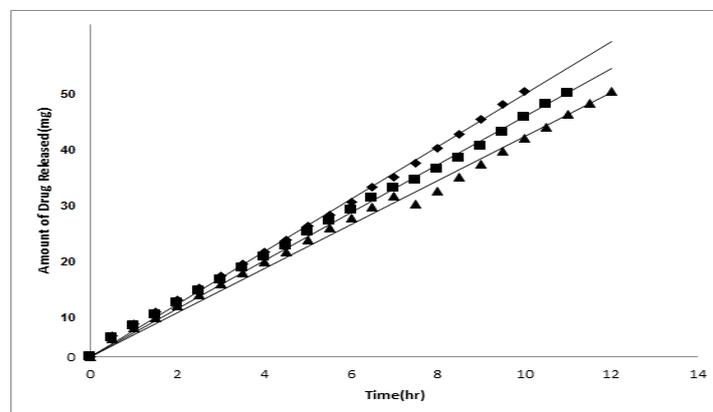
| Time (hrs.) | % of drug release |                |                |
|-------------|-------------------|----------------|----------------|
|             | F <sub>1</sub>    | F <sub>2</sub> | F <sub>3</sub> |
| 0.5         | 0                 | 0              | 0              |
| 6.5         | 16.97±0.04        | 15.95±0.03     | 14.94±0.05     |
| 12.5        | 73.14±0.04        | 66.70±0.03     | 64.76±0.03     |
| 17          |                   |                | 99.42±0.03     |
| Time (hrs.) | % of drug release |                |                |
|             | F <sub>4</sub>    | F <sub>5</sub> | F <sub>6</sub> |
| 0.5         | 0                 | 0              | 0              |
| 6.5         | 25.06±0.04        | 22.51±0.02     | 19.14±0.03     |
| 12.5        | 85.05±0.05        | 75.30±0.02     | 68.29±0.05     |
| 16          |                   |                | 99.85±0.03     |
| Time (hrs.) | % of drug release |                |                |
|             | F <sub>7</sub>    | F <sub>8</sub> | F <sub>9</sub> |
| 0.5         | 0                 | 0              | 0              |
| 6.5         | 18.17±0.02        | 17.41±0.02     | 16.84±0.03     |
| 12.5        | 88.53±0.04        | 76.38±0.03     | 72.50±0.05     |
| 15          |                   |                | 99.28±0.03     |

**Fig: 4 Comparative *In-vitro* drug release profile plot of core in cup tablets of Azathioprine prepared with Gum kondagogu in different ratios**



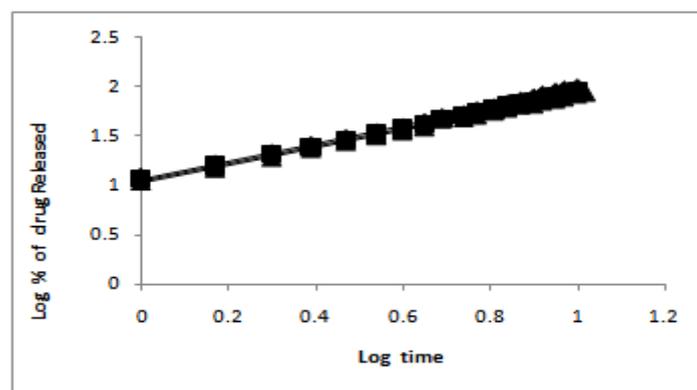
(-■-)F<sub>1</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
 (-◆-)F<sub>2</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
 (-×-)F<sub>3</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer

**Fig: 5 Comparative Zero order plots of Azathioprine core in cup tablets prepared with Gum kondagogu in different ratios**



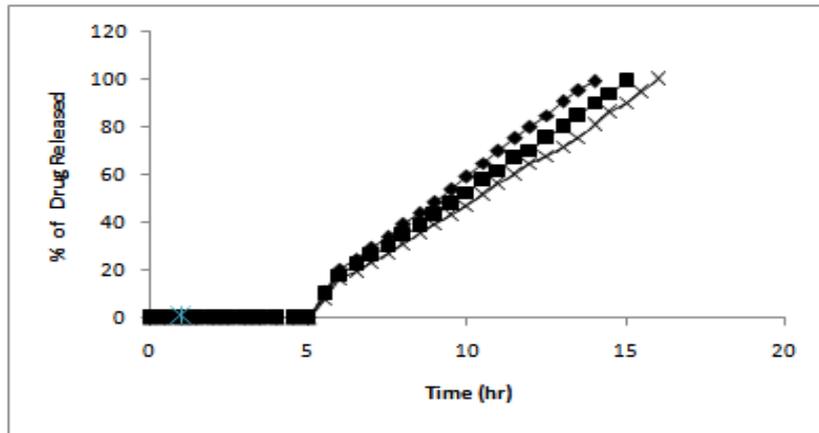
(-◆-)F<sub>1</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
 (-■-)F<sub>2</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
 (-▲-)F<sub>3</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

**Fig: 6 Comparative Peppas plots of Azathioprine core in cup tablets prepared with Gum kondagogu in different ratios**



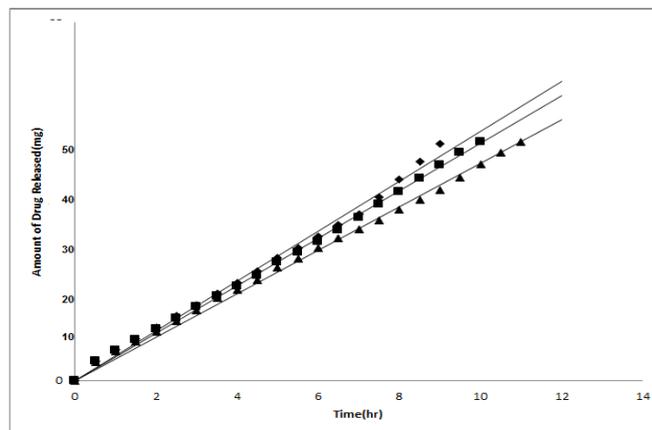
(-◆-)F<sub>1</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
 (-■-)F<sub>2</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
 (-▲-)F<sub>3</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

Fig: 7 Comparative *In-vitro* drug release profile plot of core in cup tablets of Azathioprine prepared with Gum karaya in different ratios



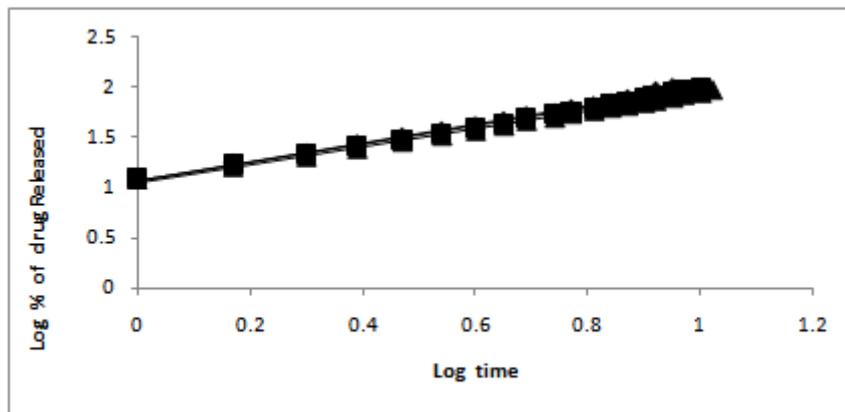
(-■-)F<sub>4</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
(-◆-)F<sub>5</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
(-×-)F<sub>6</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

Fig: 8 Comparative Zero order plots of Azathioprine core in cup tablets prepared with Gum karaya in different ratios



(-■-)F<sub>4</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
(-◆-)F<sub>5</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
(-▲-)F<sub>6</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

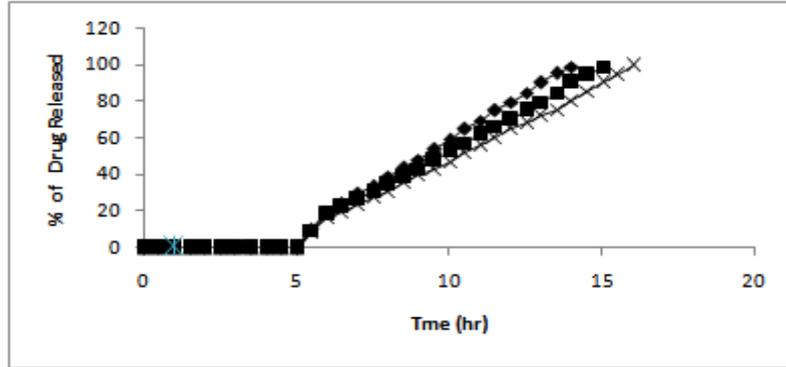
Fig: 9 Comparative Peppas plots of Azathioprine core in cup tablets prepared with Gum karaya in different ratios



(-■-)F<sub>4</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.

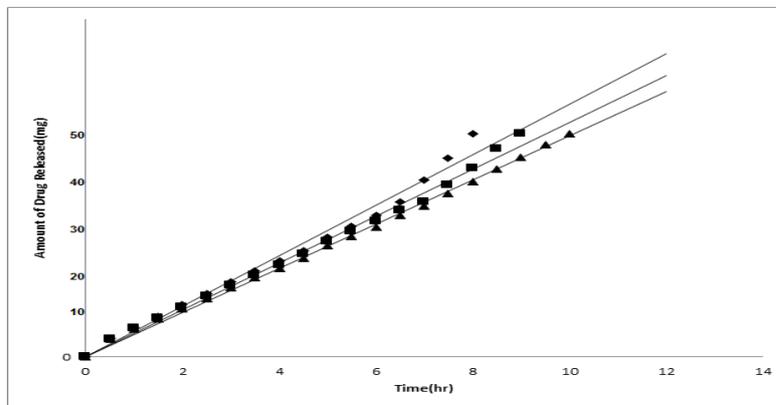
(-◆-)F<sub>5</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
 (-▲-)F<sub>6</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

**Fig: 10 Comparative *In-vitro* drug release profile consisting of Azathioprine core in cup tablets prepared with Cashew nut gum in different ratios**



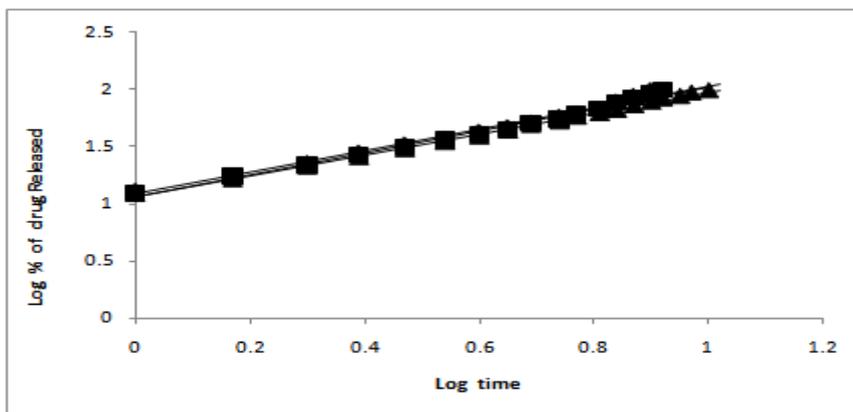
(-■-)F<sub>7</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
 (-◆-)F<sub>8</sub>: Formulation prepared with 1:1ratio of drug and polymer.  
 (-×-)F<sub>9</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

**Fig: 11 Comparative Zero order plots of Azathioprine core in cup tablets prepared with Cashew nut gum in different ratios**



(-■-)F<sub>7</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.  
 (-◆-)F<sub>8</sub>: Formulation prepared with 1:1 ratio of drug and polymer.  
 (-×-)F<sub>9</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

**Fig: 12 Comparative Zero order plots of Azathioprine core in cup tablets prepared with Cashew nut gum in different ratios**



(-♦-)F<sub>7</sub>: Formulation prepared with 1:0.5 ratio of drug and polymer.

(-■-)F<sub>8</sub>: Formulation prepared with 1:1 ratio of drug and polymer.

(-×-)F<sub>9</sub>: Formulation prepared with 1:1.5 ratio of drug and polymer.

## V. Conclusion

It is concluded from the present study that appropriate combination of a pH-dependent polymer (Eudragit S-100, R100) with a overcoat of Eudragit FS30D was suitable for adequately sustained drug release and to protect Azathioprine from being released in the upper region of the GI system. The in vitro drug release studies indicate that the optimized formulation was a promising system targeting Azathioprine to the colon. The drug release pattern from all formulations was best fitted with Higuchi release model and the drug release mechanism was followed Korsmeyer-Peppas equation with non-Fickian diffusion kinetics.

Tablet with a coating level of 10 % w/w showed a lag time of 5 hr corresponds to time required to reach colonic region. The coating polymers exhibited good results in targeting the colonic study. Formulations F3, F6, F9 were found to be more efficient in delivering the drug azathioprine to the colonic site analyzed with respect to dissolution release studies.

## Conflict of interest

Conflict of interest declared none

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