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RESEARCH PAPER

Development and Characterization of Colon Targeted Delivery System of Azathioprine

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ABSTRACT

The aim of the present work is to design and evaluates the several potential of delayed release dosage forms of Azathioprine. To prepare the multiparticulate formulation, individual wet masses were prepared for Azathioprine (150g) by employing a 1:1 ratio of the medication and polymer. Experimental procedure involved the utilisation of a total of 125g of Azathioprine, along with Chitosan and Guar gum. The process of enteric coating was carried out using a conventional coating pan, employing solutions of polymethacrylates (specifically, Eudragit L100 and Eudragit S100) at concentrations of 10-15% (w/w). The optimized batch of multiparticulate A4 filled into capsule. % Cumulative release of optimized batches C4 of capsule formulation was studied. From the coefficient determination (R2) it shows that the release of optimized batches was best fit to korsmeyer's model.

Keywords: - *Development, Characterization, Azathioprine, Colon Targeted Delivery System*

INTRODUCTION

The utilisation of colon targeted drug delivery systems shows great potential for oral administration of diverse protein, peptide, as well as biotechnological products [1-4]. This approach is particularly advantageous for substances that are susceptible to degradation by gastric proteolytic enzymes or are currently limited to intravenous administration [5-6]. For instance, the utilisation of active ingredients, such as cytotoxic drugs, which are presently limited to injectable forms, could be broadened by the advancement of colon-targeted drug delivery systems (DDS) for oral administration [7-8].

The evident advantages of oral chemotherapy include overall less cost, enhanced convenience, and better life quality of patients. Colon-targeted drug delivery systems (DDS) exhibit exceptional thermodynamic stability, extended shelf life, a notable capacity for drug solubilization, and provide effective protection against enzymatic hydrolysis [9-11].

The effectiveness of drug delivery with the pH-dependent systems is contingent upon fluctuating pH conditions within GIT, which spans a spectrum

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from extremely acidic to alkaline pH levels [12-17].

Objective of our study is to conduct a comprehensive assessment of in vitro release properties of chronotopic systems in anticipation of future advancements in this field.

The major goal of this research is to design and evaluates the several potential of delayed release dosage forms of Azathioprine to increase Patient compliance in addition to site specific drug release.

Experimental Work

Melting Point Determination: The melting point of several medications was established by placing a little amount of medication in a capillary tube that was closed at one end, placing the tube in melting point apparatus, then noting temperature at which drug melted.

Solubility Determination

Solubility of azathioprine was determined in water, ethanol, and chloroform, dilute mineral acids and dilute alkali solutions.

Loss on drying: Determination of loss on drying was conducted as per established procedure mentioned in an individual monograph. 1g of Azathioprine was dried in a vacuum at 120 ^{o}C for 6 hrs. It should not loss more than 1.0% of its weight.

Bulk density: The determination of bulk density involved the calculation of "weight of powder divided by volume of powder prior to tapping".

Tapped density: The determination of tapped density involved the calculation of "weight of powder divided by volume of powder after tapping" [18-21].

UV Spectrophotometry Studies:

Spectrophotometry is a scientific field that encompasses the measurement and quantification of the absorption of radiation energy at specific and narrow wavelengths. This typically involves the use of nearly monochromatic radiation and the analysis of chemical entities. The study employed a Shimadzu 1700 UV-visible spectrophotometer double beam, which had a scanning range of 200 to 400nm [22-23].

Standard calibration curve

For Azathioprine: The Ciprofloxacin standard stock solution was utilised to prepare working standard solutions with concentrations ranging from 05-30 µg/ml. This was achieved by pipetting suitable aliquots from stock solution into 10 ml volumetric flasks then subsequently diluting them using methanol. The absorbance values for the solutions were recorded at a wavelength of 281 nm. Subsequently, a calibration curve was made by plotting absorbance values against corresponding concentrations [22-23].

Differential scanning calorimetry (DSC): Molecular state of medication was assessed through utilisation of differential scanning calorimetry (DSC) analysis. This involved examination of both pure

azathioprine in addition to a mixture of azathioprine-pectin using a DSC-60 instrument manufactured by Shimadzu. The samples underwent heating in sealed aluminium pans within a temperature ranging between 100-3500°C, with a constant heating rate of 10.00°C per min. This process was one under a nitrogen purge of 20 ml per minute.

Preparation of Multiparticulate: The process of producing multiparticulate through Extrusion and Spheronization encompasses three fundamental steps. Firstly, the dry powder components are mixed with a liquid to create a uniformly wetted mass that is homogeneous. Secondly, the wet mass is extruded into cylindrical strands resembling spaghetti. Lastly, spheronization is employed to break down the strands into shorter cylindrical lengths and shape them into spherical forms.

To prepare the multiparticulate formulation, individual wet masses were prepared for Azathioprine (150g) by employing a 1:1 ratio of the medication and polymer. Experimental procedure involved the utilisation of a total of 125g of Azathioprine, along with Chitosan and Guar gum. The mixture was combined with varying quantities of granulating liquid, specifically demineralized water. The drug and polymer mixture was combined in a planetary mixer for a duration of 30 minutes, incorporating the necessary amount of demineralized water to create a wet mass.

Wet mass was then subjected to prepared extruded using a rotary gear extruder (Kalvika all purpose unit, Mumbai) with a cylindrical die of 14 cm length and sieve opening 1mm, screen thickness 3.25 mm, 15 rpm extrudate cut off at a length of approximately 2–3 mm.

Extruded was than spheronized in a spheronizer (Kalvika all purpose unit, Mumbai)at 1000 rpm with 10 min residence time. The multiparticulate obtained was subjected to drying in a fluidized bed dryer at a temperature of 30°C until loss on drying was less than 1.0% for Azathioprine [24-25].

Coating of multiparticulate:

The process of enteric coating was carried out using a conventional coating pan, employing solutions of polymethacrylates (specifically, Eudragit L100 and Eudragit S100) at concentrations of 10-15% (w/w). A 1:1 ratio of Eudragit S100 to Eudragit L100 was used in this study. Solutions of polymethacrylates were prepared at a concentration of 10% (w/w) in a mixture of aceton and water with a ratio of 9:1. The solution underwent plasticization using castor oil at a concentration of 20% (w/w) relative to the dry polymer. Additionally, titanium dioxide was incorporated at a concentration of 0.05% (w/v), followed by the addition of talc as a glidant at a concentration of 5% (w/w) relative to the dry polymer. Prior to utilisation, the enteric-coating dispersion underwent filtration using a 0.3-mm sieve. During the coating procedure, the coating dispersion was agitated using a magnetic stirrer. The film-coating process was conducted with specific parameters. These parameters included a pan rotating speed of 20 revolutions per minute, an atomizing air pressure of 2 bar, an inlet air temperature ranging from 60 to 70 degrees Celsius, an outlet air temperature ranging from 35 to 40 degrees

Celsius, a multiparticulate bed temperature of 38 degrees Celsius, and the application of the coating solution through a spray nozzle with a diameter of 1.1 millimetres. The film-coated multiparticulate was retained in the pan until the desired weight gain was fully attained. The multiparticulate samples were stored in vacuum desiccators at ambient temperature until they were utilised. A range of coated products with varying film thicknesses were fabricated through the manipulation of the quantity of coating solution applied, which was subsequently quantified by the percentage total weight gain (%TWG).

Table 1: Formula of Azathioprine

Batch Code	VariableLevel A	VariableLevel B	VariableLevel C	Chitosan (mg)	Guar gum(mg)	Coat Composition (%)
C1	1	-1	0	170	80	12.5
C2	1	1	0	170	170	12.5
C3	0	1	-1	130	170	10
C4	1	0	-1	170	130	10
C5	0	0	0	130	130	12.5
C6	0	0	0	130	130	12.5
C7	1	0	1	170	130	15
C8	-1	0	1	80	130	15
C9	0	0	0	130	130	12.5
C10	0	0	0	130	130	12.5
C11	-1	1	0	80	170	12.5
C12	0	-1	1	130	80	15
C13	0	1	1	130	170	15
C14	0	0	0	130	130	12.5
C15	-1	0	-1	80	130	10
C16	-1	-1	0	80	80	12.5
C17	0	-1	-1	130	80	10

Evaluation of Multiparticulate

Micromeritics studies of multiparticulates

Micromeritics properties of multiparticulates were assessed, including “Carr’s compressibility index, tapped density, bulk density, Hausner ratio, flow property, and particle size”.

Particle size determination: Determination of particle size was conducted through the utilisation of an optical microscope operating under standard polarised light conditions. Mean particle size was subsequently determined by assessing a sample of 50-100 particles, employing a calibrated ocular micrometre for accurate measurements.

Determination of bulk densities, tapped densities, and angle of repose:

The determination of bulk density involved calculation of ratio amongst mass of a powder in addition to its corresponding bulk volume, measured in cubic centimetres. Specimen, consisting of approximately 10 cubic centimetres of powder, was meticulously inserted into a graduated cylinder with 25 millilitres volume capacity. Cylindrical object was subjected to a repeated dropping experiment, with a time interval of 2 seconds, onto a rigid wooden surface. This process was

conducted three times, with the initial height of the cylinder set at 1 inch. "Determination of bulk density of individual preparation was accomplished by dividing mass of sample in grammes by final volume in cubic centimetres of sample in cylinder. Calculation was performed utilising the equation provided underneath:

$$Df = M / Vp$$

here,

Df = bulk density

M = weight of samples in grams

Vp = final volume of granules in cubic centimeter".

Determination of tapped density involved calculation of ratio amongst mass of a powder as well as corresponding volume after tapping, measured in cubic centimetres. Specimen, consisting of approximately 10 cubic centimetres of powder, is meticulously inserted in a graduated cylinder with 25 millilitres volume capacity. Experiment involved dropping a cylinder on a hard wooden surface for hundred times, with a time interval of 2 seconds between each drop. The cylinder was dropped from 1 inch height. "Tapped density of individual preparation was determined by dividing mass of sample in grammes by final volume in cubic centimetres of sample in cylinder. Calculation was performed utilising the equation provided underneath:

$$Do = M / Vp$$

here,

Do = bulk density

M = weight of samples in grams

Vp = final tapped volume of granules in cubic centimeter".

"Angle of Repose (θ), which represents flow property of multiparticulates and quantifies level of resistance to particle flow, was determined as.

$\tan \theta = 2H / D$"

Surface area of freestanding height of multiparticulates heap, denoted as 2H/D, was measured following the flow of multiparticulates from glass funnel.

Swelling ratio of multiparticulates

A predetermined mass (100 mg) of multiparticulate material devoid of any active pharmaceutical ingredient (API) was introduced into a phosphate buffer solution with a pH of 7.4. The mixture was then subjected to a swelling process for the specified duration at a temperature of 37± 0.5°C, utilising USP dissolution apparatus equipped with a dissolution basket assembly operating at a rotational speed of 100 revolutions per minute (rpm). Multiparticulate particles were periodically extracted, dried using filter paper, then their weight variations were measured throughout swelling process until equilibrium

was reached. Weight of swollen multiparticulate particles was measured following a 4-hour time interval, and subsequently, swelling ratio (SR) was computed using provided formula:

$$SR = \frac{We - Wo}{Wo}$$

Initial weight of dry multiparticulate, denoted as Wo, and the weight of swollen multiparticulate at equilibrium swelling, denoted as We, were mentioned in media. The experiment was conducted in triplicate, then mean value and standard deviation were calculated to determine SR value.

% yield of multiparticulates formed:

Determination of the % yield of multiparticulate is achieved through the process of weighing after drying. Weight of prepared multiparticulates was quantified and then divided by combined weight of all non-volatile components utilised in their preparation. This calculation yielded overall % yield of the multiparticulates.

$$\% \text{ Yield} = \left(\frac{\text{Actual weight of product}}{\text{Total weight of excipient and drug}} \right) \times 100$$

Drug entrapment determination:

For Azathioprine: A dosage of 100mg of Ciprofloxacin was administered, and the quantity of drug entrapped was determined through the process of crushing multiparticulates then subsequently extracting them in 100 ml of methanol. Following a 24-hour period, extract was subsequently moved into a volumetric flask having 100 ml capacity, and volume was adjusted by adding methanol. Solution underwent filtration, and subsequent measurement of absorbance was conducted through spectrophotometry at a wavelength of 281 nm, following appropriate dilution. Methanol was employed as a blank for comparison.

Quantity of drug encapsulated within multiparticulates was determined using subsequent mathematical equation.

$$\% \text{ Drug entrapment} = \left(\frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \right) \times 100$$

Placebo multiparticulates were utilized for reference.

***Ex-vivo* mucoadhession study**

“Evaluation of the mucoadhesive property of multiparticulate was conducted on goat's intestinal mucosa utilizing phosphate buffer, in accordance with the monograph. The multiparticulate samples were carefully distributed onto moistened tissue specimens and promptly placed onto arm of a USP tablet disintegrating test machine, with appropriate support, at a temperature of 37°C. The mass of the

multiparticulate material that was leached out was measured at various time intervals. The percentage of mucoadhesion was determined utilizing subsequent equation.

$$\% \text{ Mucoadhesion} = \frac{Wa - W1}{Wa \; x \; 100}$$

Where,

Wa = weight of multiparticulate material that has been applied.

W1 = weight of multiparticulate that has been leached out.

***In-vitro* drug release of multiparticulates of Azathioprine**

The in-vitro dissolution test was performed using the USP 2 apparatus. A sample of 1 gramme of coated multiparticulate material was placed in a rotating basket, which was set to rotate at a speed of 50 revolutions per minute. The temperature of the system was maintained at 37±0.5 degrees Celsius. The sampling procedure involved taking measurements at predetermined time intervals, and subsequently estimating medication content after appropriate dilution utilizing a double beam UV-VIS spectrophotometer. The drug release experiments were initially performed using a 900 ml solution of 0.1N hydrochloric acid (HCl) for a duration of 2 hours. This was followed by a subsequent 3-hour period using a 900 ml solution of potassium phosphate buffer with a pH of 6.8. Subsequently, a volume of 900 ml of a potassium phosphate buffer solution with a pH of 7.4 was utilised for the remaining duration of the experiment (92). The samples underwent filtration and were subsequently subjected to analysis using ultraviolet spectrophotometry at a wavelength of 281 nm. Concentration of each sample was determined using a pre-established calibration curve for Azathioprine.

Accelerated stability study of optimized batches of multiparticulate

Stability of active component is a crucial factor in determining the acceptance or rejection of dosage forms for drugs in both design and evaluation processes. Throughout stability studies, product was subjected to standard circumstances of humidity and temperature. Though, conducting study over a long duration would be more suitable in order to ensure comprehensive results. Therefore, it would be advantageous to conduct accelerated stability studies, wherein product is subjected to extreme temperature environments for a shorter time. In current stability study, multiparticulate was subjected to storage conditions of 40±2°C / 75±5% RH for 3 months. Samples were withdrawn at intervals of one, two, as well as three months for the purpose of conducting drug content analysis, swelling studies, in-vitro wash-off tests, in addition to drug release studies.

Formulation of capsule dosage form of optimized batch

Size 2 hard gelatin capsules were utilised for filling. Total of 20 capsules were prepared for each formulation using a manual method. The cap and body of the capsule with a predetermined weight were manually separated. A physical mixture with a mass of 50mg, consisting of the drug

azathioprine, was encapsulated. The residual volume of the capsule body was filled with inert starch DCP granules and compressed firmly using a glass plunger. Ultimately, the cap was securely fastened onto the body of the capsule and subsequently placed within a densely packed container for subsequent examination and analysis.

Evaluation of capsule

The optimized batch of multiparticulate A4 filled into capsule was evaluated for the following parameter

Identification attributes: The 10 capsules were randomly selected and checked for lockablity, texture, pinholes, deformity and appearance.

Weight variation: Ten capsules were selected in a random manner, and their weight was measured utilizing a single pan electronic balance. Mean weight of capsules was also determined.

Content uniformity:

10 capsules were accurately weighed each for Azathioprine (C4), remove the hard gelatin shells and empty the content and dissolved it in a methanol. The quantification of drug content was performed through measurement of absorbance at a specific wavelength of 281 nm for Azathioprine.

In-vitro drug release:

Dissolution study was conducted employing rotating basket method, as outlined in the United States Pharmacopoeia (USP), utilising the Electro lab tablet dissolution tester. A single capsule was introduced into the designated container and subsequently submerged in dissolution medium, keeping a temperature of 37±0.5°C. Great care was taken to prevent the formation of air bubbles during this process. The basket containing the capsule was then rotated at a speed of 50 revolutions per minute. The sampling procedure involved collecting samples at predetermined time intervals, which were then subjected to estimation of medication content after appropriate dilution utilizing a double beam UV-VIS spectrophotometer. The medication release experiments were initially performed in a 900 ml solution of 0.1N hydrochloric acid (HCl) for a duration of 2 hours. This was followed by a subsequent 3-hour period in which the drug release studies were conducted in a 900 ml solution of potassium phosphate buffer with a pH of 6.8. Subsequently, a volume of 900 ml of a potassium phosphate buffer solution with a pH of 7.4 was utilised for the remaining duration. The samples underwent filtration and were subsequently subjected to ultraviolet spectrophotometry at a wavelength of 281 nm in order to determine the concentration of azathioprine.

Results and Discussion

Pre-formulation Results of Selected Drug

Melting point determination

The USP pharmacopoeial limit of melting point for Azathioprine was reported to be 243-245ºC. The experimental values for given sample of drug was found to be 243.5 ºC.

Solubility Determination

Azathioprine exhibited limited solubility in water, ethanol, and chloroform, with higher solubility observed in dilute mineral acids and dilute alkali solutions.

Loss on drying:

The USP Pharmacopoeial limits for LOD of azathioprine reported to be not more than 1.0%. The experimental values for given sample of drug was found to be 0.8% signifying good agreement between the reported and experimental value.

Bulk density:

Bulk density of azathioprine was found 0.454 gm/cm^3.

Tapped density:

Tapped density of azathioprine was found 0.555 gm/cm^3.

UV spectrophotometry studies:

Appropriate dilutions was prepared from standard stock solution of drugs azathioprine then scanned in spectrum mode from 400-200 nm. Drug displayed absorbance maxima at 281nm.

Standard calibration curve

Azathioprine showed maximum absorbance at 281 nm wavelength (λ_{max}).. Further analysis was done at this wavelength. Plot of absorbance against concentration was found to be a straight line (R^2 = 0.999). This was in accordance with Beers-Lamberts law. Equation of regressed line was found y = 0.122x + 0.049. Drug showed molar absorptivity of 138.26 Lmol^{-1}cm^{-1}. This absorptivity value indicates that drug can be analyzed efficiently using UV absorption spectroscopy.

Table 2: Standard calibration of Azathioprine

Conc. (µg/ml)	Abs.* at 281 nm
5	0.4148± 0.0062
10	0.7896± 0.0053
15	1.1493± 0.0052
20	1.5191± 0.0056
25	1.9118± 0.0012
30	2.2449± 0.0081

***Values are in triplicate (n=3)**

Differential scanning calorimetry (DSC):

Molecular condition of pharmaceutical compound was evaluated through the implementation of a differential scanning calorimetry (DSC) on both unadulterated azathioprine azathioprine and a

composite of azathioprine and polymers. The analysis was conducted utilising a differential scanning calorimeter (DSC-60, Shimadzu). Specimens underwent heating in hermetically sealed aluminium containers within a temperature range of 10°C to 350°C. This process was carried out at a consistent rate of 10.0°C per minute, while a nitrogen flow of 20 ml per minute was used to remove any impurities. The peak of azathioprine was observed at a temperature of 245°C, as depicted in both figures. Additionally, the peak of pectin was observed at a temperature of 160°C. Based on the depicted selection of azathioprine in both figures, it can be inferred that there is an absence of interaction amid polymers and Azathioprine.

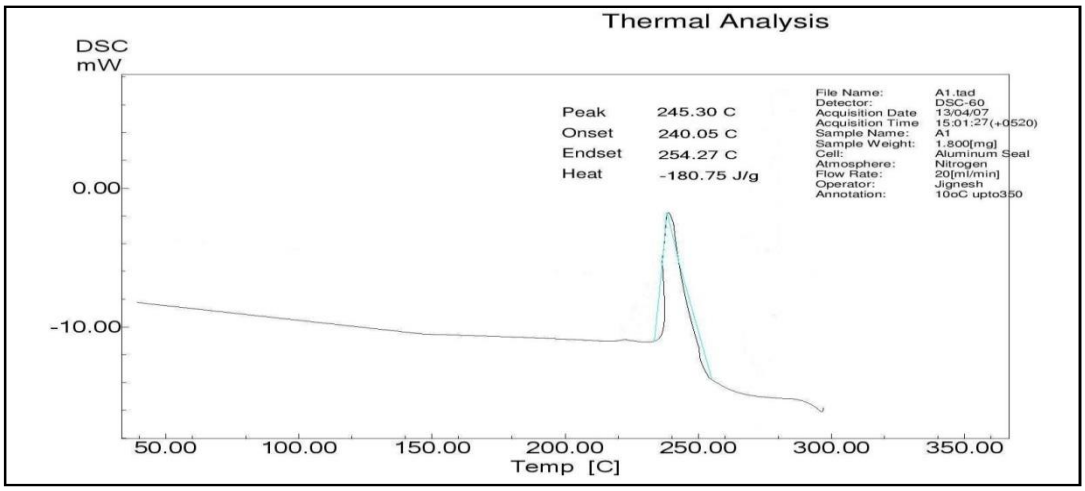

Figure 1: DSC of Azathioprine

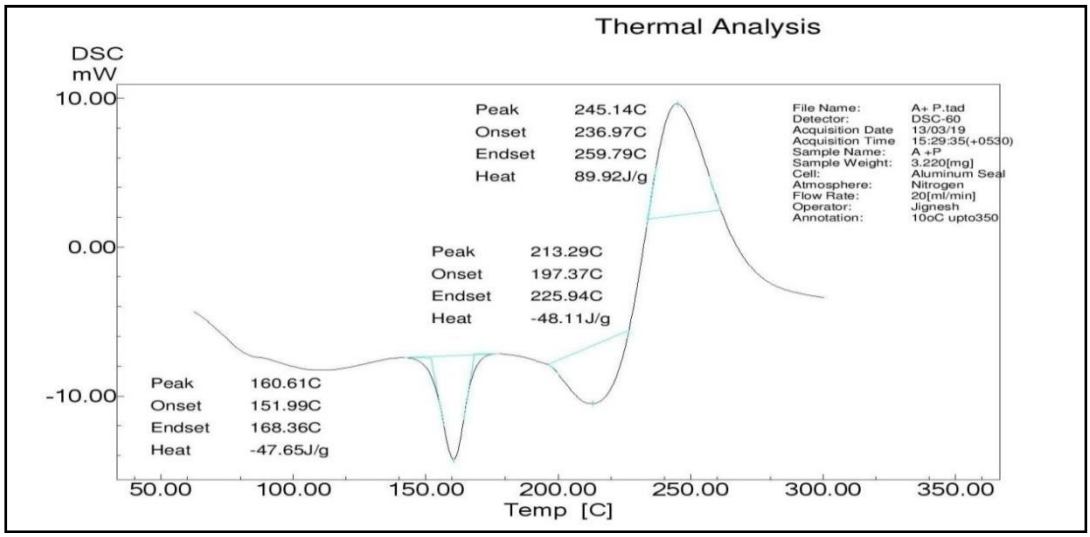

Figure 2: DSC of Azathioprine + Polymers

Evaluation for Multiparticulate

Micromeritic studies of Multiparticulate

The average particle size of the factorial batches was between one and two millimetres, and the measured density of the azathioprine was between half a gramme and a half a gramme per cubic centimetre. It was discovered that all of the factorial batches had a bulk density that fell somewhere

between 0.48-0.62 g/cm^3. Angle of repose was determined to be within 25° to 35° C, which was an appreciable limit for multiparticulate to display flow property. Every formulation demonstrated good flowability in this regard, as indicated in terms of the angle of repose.

Table 3: Micromeritic studies of factorial batches of Azathioprine

Parameters Batches	Average particle size (mm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose (°)
C1	1.2 ± 0.054	0.50 ± 0.05	0.53±0.05	31.24±1.2
C2	1.1 ± 0.015	0.50 ± 0.04	0.51±0.03	29.54±1.4
C3	1.3 ± 0.012	0.50 ± 0.03	0.53±0.05	30.21±1.3
C4	1.2 ±0.015	0.52 ± 0.06	0.55±0.06	29.52±1.2
C5	1.5 ± 0.013	0.53 ± 0.04	0.51±0.08	28.51±1.2
C6	1.2 ± 0.014	0.52 ± 0.06	0.53±0.03	29.74±1.2
C7	1.4 ± 0.084	0.49 ± 0.04	0.50±0.08	30.46±1.3
C8	1.2 ± 0.014	0.53 ± 0.04	0.51±0.08	30.41±1.2
C9	1.2 ± 0.078	0.49 ± 0.02	0.51±0.03	32.32±1.1
C10	1.5 ±0.012	0.48 ± 0.04	0.53±0.07	28.54±1.2
C11	1.4 ± 0.056	0.52 ± 0.07	0.54±0.04	29.74±1.4
C12	1.6 ± 0.033	0.50 ± 0.05	0.53±0.05	29.82±1.3
C13	1.5 ± 0.053	0.51 ± 0.02	0.55±0.03	30.21±1.4
C14	1.5 ± 0.022	0.48 ± 0.03	0.51±0.02	29.63±1.2
C15	1.5 ± 0.045	0.50 ± 0.07	0.53±0.06	31.35±1.1
C16	1.5 ± 0.023	0.52 ± 0.01	0.55±0.04	29.56±1.4
C17	1.4 ± 0.054	0.52 ± 0.08	0.53±0.05	30.31±1.3

***Three separate analyses were performed on each sample (n = 3)**

Swelling ratio of multiparticulate:

It was discovered that the swelling ratio of the factorial batches of azathioprine C1, C2, C4, and C7 was higher in comparison to other formulations; among these four batches, batch A4 displays the largest swelling, which is 1.810.15 percent. One possible explanation for this is because the formulation contains a higher concentration of chitosan. At first, each of the batches expands gradually, but eventually they reach their maximum size relative to the other concentrations listed in the table 4.

Table 4: Swelling ratio of factorial batches of Azathioprine

Batch Code	Swelling ratio of multiparticulate adhering to tissue							
	In pH 7.4							
	Time/h							
	0	**1**	**2**	**4**	**6**	**8**	**10**	**12**
C1	0	0.42±0.13	0.52±0.13	0.64±0.16	0.75±0.12	0.98±0.16	1.29±0.15	1.68±0.12
C2	0	0.45±0.14	0.54±0.14	0.62±0.15	0.94±0.15	1.24±0.16	1.34±0.12	1.62±0.17
C3	0	0.43±0.13	0.65±0.15	0.76±0.15	0.84±0.13	0.92±0.13	1.35±0.15	1.62±0.12
C4	0	0.48±0.15	0.54±0.18	0.68±0.18	0.86±0.14	1.36±0.15	1.61±0.13	1.81±0.15
C5	0	0.42±0.12	0.57±0.17	0.57±0.15	0.74±0.15	0.94±0.14	0.94±0.15	1.61±0.15
C6	0	0.45±0.13	0.58±0.16	0.73±0.14	0.87±0.17	0.98±0.13	1.27±0.13	1.53±0.13
C7	0	0.55±0.14	0.52±0.13	0.78±0.15	0.93±0.16	1.31±0.16	1.42±0.12	1.68±0.14
C8	0	0.48±0.15	0.62±0.14	0.75±0.14	0.83±0.15	0.92±0.15	1.25±0.14	1.55±0.15
C9	0	0.57±0.14	0.58±0.14	0.72±0.12	0.87±0.14	1.26±0.13	1.44±0.17	1.52±0.15
C10	0	0.46±0.14	0.56±0.15	0.68±0.15	0.75±0.16	1.23±0.11	1.42±0.16	1.53±0.14
C11	0	0.49±0.15	0.61±0.14	0.74±0.15	0.85±0.13	0.96±0.12	1.19±0.14	1.60±0.12
C12	0	0.47±0.13	0.54±0.15	0.63±0.13	0.92±0.19	1.23±0.16	1.36±0.15	1.56±0.18
C13	0	0.54±0.13	0.65±0.15	0.76±0.16	0.84±0.17	0.92±0.13	1.32±0.16	1.62±0.12
C14	0	0.58±0.12	0.54±0.13	0.68±0.11	0.86±0.14	1.36±0.15	1.51±0.13	1.64±0.12
C15	0	0.42±0.12	0.57±0.14	0.67±0.14	0.74±0.15	0.84±0.14	0.94±0.15	1.51±0.15
C16	0	0.45±0.16	0.58±0.13	0.63±0.16	0.87±0.15	0.94±0.13	1.27±0.18	1.58±0.13
C17	0	0.55±0.15	0.52±0.12	0.78±0.15	0.98±0.14	1.34±0.16	1.42±0.16	1.54±0.14

***Three separate analyses were performed on each sample (n = 3)**

% yield of multiparticulate

An excellent percentage yield for azathioprine was also shown for the prepared factorial batches C1, C2, C4, and C7 (91.20 1.22%, 93.83 2.14%, 95.12 2.15%, and 94.16 1.15%, correspondingly), which compares favourably to the results of the other batches. For factorial batches also, batch C4 showed increase in percentage yield of multiparticulate compared to other this may be because of high viscosity as well as molecular weight of polymer Chitosan in addition to Guar gum as mentioned in table 5.

Table 5: Percentage yield of factorial batches of Azathioprine

Batch Code	% Yield
C1	91.20± 1.22
C2	93.83± 2.14
C3	92.58± 1.14

C4	95.12± 2.15
C5	94.32± 2.45
C6	91.16± 2.12
C7	94.16± 1.15
C8	91.55± 1.14
C9	93.62± 1.32
C10	92.30± 2.13
C11	92.16± 1.15
C12	91.55± 1.12
C13	93.62± 1.42
C14	92.30± 1.13
C15	91.20± 1.22
C16	93.43± 2.14
C17	91.58± 1.14

*** Three separate analyses were performed on each sample (n = 3)**

Drug entrapment study

Drug entrapment of factorial batches

Prepared A considerable percentage of the medicine is also entrapped in the factorial batches of multiparticulate, which range from 60 to 80 percent. For azathioprine, batch A4 has a higher percentage of entrapment—80.24 plus or minus 2.12%—than the other formulation. It was evident from these findings that an upsurge in polymer concentration, particularly Chitosan, results in an upsurge in the amount of medicine that is entrapped, as shown in table 6.

Table 6: % Drug entrapped of factorial batches of Azathioprine

Batch No	Drug entrapment efficiency	
	Drug Conc. (mg)	% Drug entrapment
C1	150	64.71± 1.42
C2	150	66.42± 1.12
C3	150	62.51± 3.22
C4	150	80.24± 2.12
C5	150	79.32± 1.52
C6	150	79.64± 1.32
C7	150	80.02± 2.13
C8	150	75.62± 1.11
C9	150	79.51± 1.42
C10	150	79.64± 1.22
C11	150	77.52± 1.32
C12	150	60.52± 1.12
C13	150	62.34± 1.17
C14	150	79.34± 1.42
C15	150	75.22± 1.16
C16	150	63.59± 1.32
C17	150	61.42± 1.32

***Three separate analyses were performed on each sample (n = 3)**

The drug entrapment of multiparticulate increased greatly from about 60 to about 84% as drug/polymer ratio enhanced from 1:1 to 2:1. It also shows a proportional increase in drug loading efficiency of Chitosan: Guar gum multiparticulate at enhanced concentrations of Chitosan in the multiparticulate preparative mixture.

In-vitro drug release studies

For factorial batches dissolution study was carried out and sample were analyzed after 2nd, 4th, 6th, 9th, 12th, 18th and 24th h. The results showed that azathioprine batch C4 releases maximum of medication i.e. 97.64±2.15%, as related to other formulations.

Table 7: % Cumulative release of factorial batches of Azathioprine

Batch Code		Time / h						
	0	**2**	**4**	**6**	**9**	**12**	**18**	**24**
C1	0	19.43±2.31	28.71±2.43	30.53±2.12	75.46±2.03	80.14±2.15	87.46±2.25	95.24±3.21
C2	0	19.62±3.52	29.06±3.43	30.23±2.32	77.63±2.18	85.62±2.24	89.67±2.13	95.86±2.23
C3	0	18.96±3.45	28.92±2.32	30.82±2.52	70.56±3.24	79.56±3.27	84.57±2.30	89.57±2.13
C4	0	17.81±2.12	28.33±2.52	30.24±3.32	79.65±2.22	86.72±2.23	91.28±2.52	97.64±2.15
C5	0	18.67±3.41	28.43±2.62	28.76±2.41	70.11±2.28	78.85±2.26	83.74±2.21	87.9±0312
C6	0	18.72±3.42	27.98±3.41	29.76±2.23	70.2±3.25	79.11±2.25	83.69±2.40	87.54±2.09
C7	0	19.23±3.14	29.44±2.43	30.32±3.42	79.56±2.21	86.35±3.24	90.42±2.31	95.62±2.13
C8	0	18.22±2.32	28.67±2.52	28.44±2.32	69.31±2.09	76.31±2.13	82.47±3.22	93.51±3.26
C9	0	18.54±2.56	28.53±3.16	29.59±2.22	70.13±2.27	79.18±3.15	83.77±2.60	87.92±2.71
C10	0	18.82±2.32	28.63±2.52	30.01±2.41	70.24±2.22	79.24±2.14	83.71±3.18	87.25±2.43
C11	0	17.43±2.51	27.62±2.37	30.31±3.43	69.24±2.22	78.64±2.20	82.11±2.14	93.87±2.28
C12	0	17.54±2.43	27.68±3.31	30.32±2.42	64.21±2.11	70.05±2.24	83.2±2.25	90.52±2.42
C13	0	17.52±3.52	28.06±2.52	29.97±2.41	68.21±3.08	70.26±3.14	84.63±2.28	89.48±2.32
C14	0	18.59±2.35	28.91±2.38	30.06±3.43	70.26±2.50	79.15±2.20	83.69±2.13	87.41±3.08
C15	0	17.20±3.12	27.83±2.43	29.88±2.52	60.28±2.29	77.84±2.23	82.31±3.15	93.08±2.20
C16	0	18.20±3.42	27.52±3.43	30.34±2.22	68.34±3.23	73.54±2.15	80.16±3.12	88.67±2.38
C17	0	18.40±2.32	27.89±2.42	28.97±2.32	61.02±2.21	68.65±2.12	83.07±2.23	90.2±2.24

***Three separate analyses were performed on each sample. (n = 3)**

Statistical analysis

After determining slope of the suitable equations, correlation coefficient (R) was then calculated for each of formulations. This allowed release rate constant to be determined. When compared to other formulations, it was discovered that release profile as well as entrapment efficiency of the formulation of factorial batches C4 for azathioprine were satisfactory.

The in-vitro drug release of C4 was best explained by the k-peppas equation, which had highest linearity R2 values at 0.9979, 0.9904, and 0.9966. This was followed by the Higuchi equation, which had R2 values at 0.9944, 0.9833, and 0.9850, and First order R2 values at 0.9811, 0.9821, and 0.9852 correspondingly. This suggests that the pharmaceutical agent spread from the polymeric matrix. It was discovered that release of medication closely followed the Higuchi kinetics, which suggests that medication diffuses at a somewhat slow pace as distance of diffusion rises. In addition, value of 'n' in the Korsemeyer-Peppas equation for C4 is 0.8054, which specifies a purely relaxed regulated

delivery. This type of transport was referred to as Case II. On occasion, values of n that are more than 0.89 have been discovered, which were previously considered to be super case II kinetics. Because of the linkage of the diffusion process with mechanical reaction of polymer chitosan along with guar gum, outcomes of this study unequivocally support non-fickian model of diffusion.

Assessment of difference factor and similarity factor for optimized batches

After three months of storage in accelerated circumstances (400C ±20C and 75% ±5% RH), the optimised formulation for azathioprine (C4) was tested to determine difference factor (f1) and similarity factor (f2) of dissolving rate research. When calculating dissimilarity factor (f1) and the similarity factor (f2), dissolution profile of the formulation in its first stage was used as a reference. It was determined that the two dissolution profiles (test and reference) were comparable when the value of f2 was between 50 and 100 and f1 was less than 15.

Accelerated stability study of optimized batches of multiarticulate

After three months of being subjected to an accelerated condition, the data that were obtained showed that there was no discernible alteration in the dissolving profiles of preparations. According to the findings, it was determined that the formulation may be regarded stable after being stored under accelerated stability conditions for three months.

Table 8: *In-vitro* dissolution profile of optimized formulation for one month at 40 °C ± 2 °C and 75% RH ± 5% RH.

Time/h	C4
0	0
2	17.32±1.03
4	28.12±1.38
6	30.06±1.15
9	79.40±1.40
12	86.13±1.12
18	91.07±1.18
24	97.31±1.07

***Three separate analyses were performed on each sample. (n = 3)**

Table 9: *In-vitro* dissolution profile of optimized formulation for two months at 40 °C ± 2^0C and 75% RH ± 5% RH

Time/h	C4
0	0
2	17.04±1.13
4	28.03±1.31
6	30.06±1.05
9	79.31±1.10
12	86.08±1.02
18	91.01±1.18
24	97.11±1.02

***Three separate analyses were performed on each sample. (n = 3)**

Table 10: *In-vitro* dissolution profile of optimized formulation for three months at 40 ^{0}C ± 2^{0}C and 75% RH ± 5% RH.

Time/h	C4
0	0
2	17.04±1.16
4	27.82±1.32
6	29.16±1.35
9	79.21±1.19
12	85.98±1.22
18	90.81±1.18
24	96.71±1.32

***Three separate analyses were performed on each sample. (n = 3)**

Table 11: Assessment of f1 and f2 for C4, K10 & 5FU15 batches after 3 monthsat 40^{0}C ± 2^{0}C and 75% RH ± 5% RH

Batch Code	Factors	Initial	One Month	Two Month	Three Month
C4	f_1	…	2.43	3.22	4.31
	f_2	…	89.52	77.63	73.34

***Initial sample (0 month) was taken as reference to calculate f1 and f2 values.**

Formulation of capsule dosage form of optimized batch

Capsules were efficaciously prepared for optimized batches of multiparticulate byfilling it together with dummy starch DCP granules then were exposed to assessments.

Table 12: Formulation of capsule dosage form of optimized batches

Batches Code	Drug content (mg)	%Drug entrapment	Multiparticulate filled equivalent to drug dose(mg)	Capsule size
C4	150	97.68± 2.8	550.64	2

***Three separate analyses were performed on each sample (n = 3)**

Evaluation of Capsules

Identification attributes:

All capsules were lockable with fine and smooth texture. The body of the capsules was hard and not sticking even when touched with wet finger. There were no signs of pinholes and deformity giving satisfactory appearance.

Weight variation:

Individual weights of every capsule were extremely uniform, and there was no discernible difference in weight between any of the capsules. It was discovered that the capsules in the C4 batch had an average weight of 550.64 mg each.

Content uniformity test:

The amount of drug for C4 batch in each capsule was found to be 248.32 ± 2.3 mghaving 96.32± 2.8 % drug content. Every sample was evaluated thrice (n= 3).

In-vitro drug release from capsule of optimized batches

In-vitro dissolution profile showed that cap was dissolved in 15 minutes in all of the formulations. After that, there was a larger initial release of medication, followed by a steady and almost constant release of medication over the course of 24 hours. Rate at which drug was released varied based on kind and amount of polymer that was utilised.

Table 13: % Cumulative release of optimized batches C4 of capsule formulation

Time/h	C4
0	0
2	14.20±1.24
4	20.89±1.45
6	29.73±1.31
9	73.78±1.53
12	80.88±1.45
18	88.32±1.43
24	96.84±1.11

***Three separate analyses were performed on each sample. (n = 3)**

Kinetic assessment

From the results of dissolution data by using korsmeyer's and peppas equation, it shows that optimized batches give the non-fickian release. Different kinetics was applied to interpret the release rate azathioprine from optimized batches C4. From the coefficient determination (R^2) it shows that the release of optimized batches was best fit to korsmeyer's model.

Table 14: Kinetic assessment of release data for capsule

Batch code	R^2 value of Kinetic Equation				
	Zero-order	First-order	Higuchi	Hixson-Crowell	Korsmeyer's-Peppas model
C4	0.8566	0.9194	0.8980	0.9528	0.9679

Three separate analyses were performed on each sample. (n = 3)

Conclusion

The colon targeted delivery system of azathioprine was developed. In-vitro dissolution profile showed that capsule was dissolved in 15 minutes in all of the formulations. After that, there was a larger initial release of medication, followed by a steady and almost constant release of medication over the course of 24 hours.

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