Biological Forum – An International Journal **15(5a): 492-505(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Factorial Design Formulation Optimization and *in vitro* characterization of Colon Targeted Delivery System of Azathioprine

Shikha Jakhotiya[1]* and Gajendra Singh Rathore[2]
[1]Research Scholar, Department of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan), India.
[2]Department of Pharmacy, Bhupal Nobles' University, Udaipur (Rajasthan), India.

(Corresponding author: Shikha Jakhotiya)*
(Received: 24 March 2023; Revised: 26 April 2023; Accepted: 03 May 2023; Published: 15 May 2023)
(Published by Research Trend)

ABSTRACT: Colon-targeted drug delivery systems are designed to deliver medications specifically to the colon, which can be useful for the treatment of various gastrointestinal disorders. The current work's goal is to develop and optimise an Azathioprine colon focused delivery device. Azathioprine (150g) was manufactured as separate wet masses using a 1:1 ratio of the drug to the polymer in order to create the multiparticulate formulation. As part of the experimental process, 125g of azathioprine, coupled with chitosan and guar gum, were used. Using a standard coating pan and polymethacrylate solutions (namely, Eudragit L100 and Eudragit S100 at concentrations of 10-15% (w/w) each), enteric coating was carried out. Multiparticulate C4 from the optimised batch was placed inside a capsule. The oral colon administration system is a cutting-edge method for treating various diseases. Azathioprine's enhanced colon drug delivery mechanism may be employed for future testing with a view to clinical usage.

Keywords: Formulation, Optimization, Azathioprine.

INTRODUCTION

Oral administration of various proteins, peptides, and pharmaceutical products has a lot of promise when colon-targeted drug delivery methods are used (Rajpurohit *et al*., 2010; Bourgeois *et al*., 2005; Vass *et al*., 2019). This method is especially beneficial for drugs that can only presently be administered intravenously or are subject to breakdown by stomach proteolytic enzymes (Bruno *et al*., 2013; Florek *et al*., 2017). For instance, the development of colon-targeted drug delivery systems (DDS) for oral administration might increase the use of active components, such as cytotoxic medicines, which are now only available in injectable forms (Farasati Far *et al*., 2022; Lorscheider *et al*., 2021; Pandiya & Sharma 2021).

The evident advantages of oral chemotherapy include overall less cost, enhanced convenience, and better life quality of patients (Sagar *et al*., 2021; Kumar *et al*., 2023). 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 (Ghosh *et al*., 2019; Sivamaruthi *et al*., 2022; de Sousa Victor *et al*., 2020).

The pH conditions in the GIT, which range from severely acidic to alkaline pH values, are what determine how well drugs are delivered with pH-dependent systems (Naeem *et al*., 2015; Taniguchi *et al*., 2014).

The pH conditions in the GIT, which range from severely acidic to alkaline pH values, are what determine how well drugs are delivered with pH-dependent systems (Zhang *et al*., 2020; Chen *et al*., 2020).

Azathioprine is a medication primarily used as an immunosuppressant and immunomodulatory drug. It is commonly prescribed to manage various autoimmune and inflammatory conditions (Broen *et al*., 2020; Ido *et al*., 2021; Patel *et al*., 2006).

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

MATERIAL AND METHODS

In the current research work, the colon targeted delivery system of Azathioprine was optimized.

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 100-350°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 (Remmele *et al*., 1998).

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 (Dhole *et al*., 2011; Belgamwar *et al*., 2009).

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) (Dhole *et al*., 2011; Belgamwar *et al*., 2009).

Preliminary trial batches.

Batch code for Azathioprne	Drug: Polymer Ratio wt/wt	Polymer Ratio (CH:GG) wt/wt
C1a	1:1	1:1
C2a	1:1	1:1.5
C3a	1:1	1.5:1
C4a	1:1	1:2
C5a	1:1	2:1
C1b	1:1	1:1
C2b	1:1	1:1.5
C3b	1:1	1.5:1
C4b	1:1	1:2
C5b	1:1	2:1

Experimental Design for Optimization: The utilisation of response surface methodology (RSM) is a prevalent and established practice in the field of drug delivery device development and *optimiz*ation. The methodology employed in this study is grounded in principle of design of experiments. It involves utilisation of diverse experimental designs, the generation of polynomial equations, and mapping of response across experimental domain. These approaches are employed to ascertain the optimal processing variables. The utilisation of this technique necessitates a minimal amount of experimentation and time, thereby demonstrating its superior efficacy and cost-effectiveness in comparison to traditional approaches for formulating dosage forms. The Box-Behnken design was employed to statistically *optimiz*e formulation parameters and assess primary effects, interaction effects, along with quadratic effects of process parameters related to the ratio of drug polymer as well as coat composition in enteric coated multiparticulate. Study employed a 3-factor, 3-level experimental design to investigate quadratic as well as linear response surfaces. Design Expert Software (Version 9.0.1, Stat-Ease Inc., Minneapolis, MN) was utilised for this purpose. Statistical validity of polynomials was determined by conducting an analysis of variance (ANOVA) using Design Expert software. The level of significance was set at a value of $p < 0.05$. The statistical parameters such as the coefficient of variation (CV), the multiple correlation coefficient

(R2), the adjusted multiple correlation coefficient (adjusted R2), and the predicted residual sum of squares (PRESS), which were provided by the software, were used in the process of selecting the mathematical model that was deemed to be the most appropriate. The PRESS statistic is used to evaluate the goodness of fit of a model to data. It is expected that chosen model will have a relatively small PRESS value compared to other models being considered. Design Expert software was utilised to generate both 3-D response surface graphs as well as 2-D contour plots. The utilisation of these plots proved to be highly beneficial in visualising the interaction effects of factors on the responses. An experimental design was conducted to *optimiz*e the composition of polymer along with coat (Eudragit S and L 100) for the study. The present study utilised Box-Behnken design to investigate impact of independent variables, namely Guar gum, Chitosan, as well as coat composition (Eudragit S and L 100), on dependent variables including mucoadhesion, entrapment efficiency, and % drug release at specific time points (9th h, 12th h, 18th h, and 24th h).

The table 1 presented below displays the various variables and their corresponding levels that were employed in the *optimiz*ation design. The factorial batches were designed by utilising these variables at three distinct levels.

The multiparticulate was prepared using same parameter as mentioned for trial batch having 1:1 drug: polymer ratio. The A5a, batches was selected as an optimized batch as they shows the best result as compared to other batches with polymer ratio of 2:1 (Chitosan: Guar gum w/w). The extruded was than spheronized and coating were done using same parameters as mentioned for trial batches (Akl *et al*., 2016; Elnaggar *et al*., 2009).

Table 1: Factorial batches of Azathioprine.

Batch Code	VariableLevel A	Variable Level B	Variable Level C	Chitosan (mg)	Guargum (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

Variable level: Low (-1), Medium (0), High (1)

Evaluation of Multiparticulate:

Micromeritics studies of multiparticulates:

Micromeritics properties of the 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 (Shekunov *et al*., 2007).

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

The determination of bulk density involved calculation of ratio amongst mass of a powder and 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 a volume capacity of 25 millilitres. The 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 formulation 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 the ratio between mass of a powder and 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 a volume capacity of 25 millilitres. The experiment involved dropping a

cylinder on a hard wood surface 100 times, with a time interval of 2 seconds between each drop. The cylinder was dropped from 1 inch height. Tapped density of individual formulation 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 (Shekunov *et al*., 2007).

Percentage 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.

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

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 and subsequently extracting them in 100 ml of methanol. Following a 24-hour period, extract was subsequently transferred into a volumetric flask with a capacity of 100 ml, 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 used as reference.

RESULT AND DISCUSSION

Differential scanning calorimetry (DSC). The molecular condition of the pharmaceutical compound was evaluated through the implementation of a differential scanning calorimetry (DSC) analysis on both the unadulterated azathioprine drug and a composite of azathioprine and polymers. The analysis was conducted utilising a differential scanning calorimeter (DSC-60, Shimadzu). The specimens underwent heating in hermetically sealed aluminium containers within a temperature range of 100°C to 350 °C. This process was carried out at a consistent rate of 10.00°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.

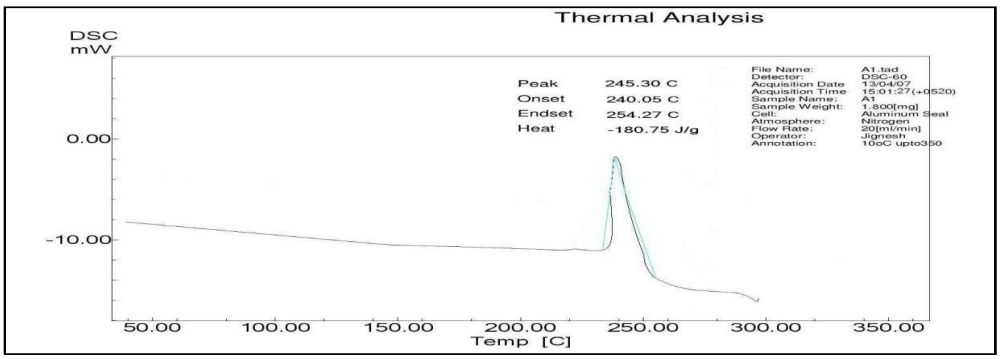

Fig. 1. DSC of Azathioprine

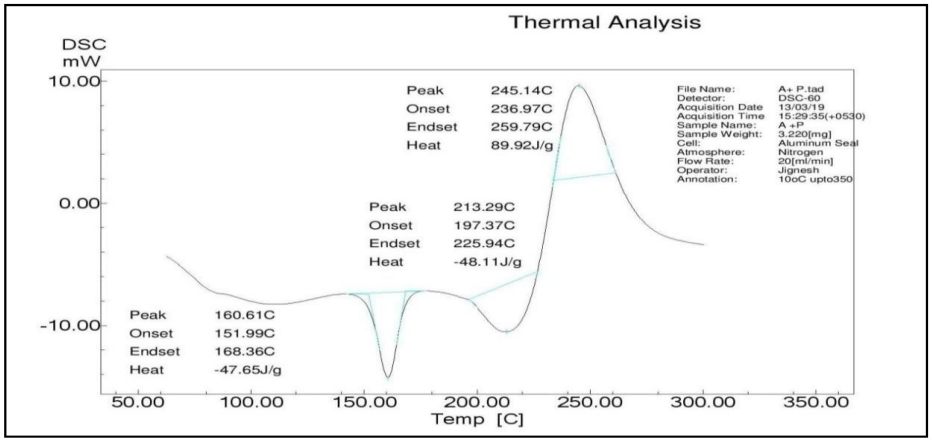

Fig. 2. DSC of Azathioprine + Polymers

Preparation of Multiparticulate: The multiparticulates were prepared successfully by extrusion and speronization method with good practical yield.

The initial trial batches were prepared and assessed, from which it was observed that Azathioprine batch C3a, C5a, C3b, and C5b exhibited favourable practical yield. Batch C3a, C3b contain 1:1 ratio of drug and polymer in which the polymers Guar gum (GG) and Chitosan (CH) concentration was taken in varied proportion as shown in Table 1. For C3a batch, the concentration of Chitosan (CH) and Guar gum (GG) was 1.5:1 (CH: GG) w/w, having 10% w/w coat composition and for C3b, the concentration of Chitosan (CH) and Guar gum (GG) was 2:1 (CH: GG) wt/wt having 15% w/w coat composition. Batch C3a demonstrated superior entrapment efficiency, swelling index, *ex-vivo* mucoadhesion, and *in-vitro* drug release compared to other formulations, thus establishing it as the *optimize*d formulation.

From factorial batches, batch C4 shows maximum practical yield respectively. C4 batch contains 170mg (Variable level A (1)) of Chitosan and 130 mg (Variable level B (0)) of Guar gum with 10% w/w (Variable level C (-1)) coat composition. Batch A4 exhibited favourable *ex-vivo* mucoadhesion, swelling index, entrapment efficiency, and *in-vitro* drug release characteristics, thereby establishing it as an *optimize*d formulation.

The aforementioned study has reached the conclusion that the combination of a high concentration of Guar gum and Chitosan leads to the retardation of drug release. The lack of disintegration of Chitosan, potentially caused by cross-linking, in the presence of colon enzymes, as well as its coating with Eudragit S and L 100, serves to impede its release in the gastrointestinal tract as well as facilitates targeted drug delivery to the colon.

Coating of Multiparticulate. In coat composition initially the plasticizer concentration was taken as 10% w/wwith respect to dry polymer and drug release was studied for 24 h. The formulated multiparticulates formed were too intact and the release rate of drug was relatively low. The plasticizer concentration then increased to 20% w/w so as to have ease in the multiparticulate preparation and get the proper release rate of drug.

Table 2: *In-vitro* drug release studies for trial batches of Azathioprine using 10% w/w plasticizer.

Batch Code	Time / h							
	0	**2**	**4**	**6**	**9**	**12**	**18**	**24**
C1a	0	4.03± 1.2	9.05± 5.4	13.22± 2.6	23.87± 1.6	34.29± 2.6	40.31± 2.6	48.61± 5.3
C2a	0	5.06± 1.4	9.11± 2.4	14.32± 3.5	24.57± 3.2	36.2± 1.6	41.46± 1.7	50.34± 3.4
C3a	0	4.11± 3.4	9.07± 1.7	13.26± 6.4	23.67± 1.3	33.19± 1.3	41.09± 3.4	50.32± 1.7
C4a	0	4.10± 2.3	9.04± 6.4	13.30± 5.2	23.52± 4.4	33.25± 5.4	40.11± 1.7	49.41± 2.5
C5a	0	5.12± 1.2	8.18± 1.3	11.22± 3.5	22.97± 6.5	32.39± 6.3	39.84± 3.8	48.81± 3.2
C1b	0	3.20± 1.4	6.12± 4.2	10.31± 1.6	20.96± 3.4	31.24± 3.8	39.78± 3.4	48.71± 1.7
C2b	0	3.35± 1.1	7.09± 1.8	11.12± 1.4	22.43± 1.4	32.42± 2.4	40.64± 6.7	48.61± 3.5
C3b	0	4.09± 3.4	7.12± 1.5	11.21± 2.3	23.15± 2.4	33.51± 1.9	40.54± 5.4	49.11± 1.8
C4b	0	3.13± 1.6	6.14± 1.4	10.22± 3.6	20.91± 1.4	31.59± 1.5	39.54± 3.2	48.56± 1.4
C5b	0	3.45± 1.4	7.13± 2.9	11.30± 1.4	23.19± 1.6	33.26± 1.4	40.24± 1.4	49.51± 1.4

The extent of the plasticizer partitioning was explored with regard to the type and concentration of the plasticizer, as well as the solids content of the polymer dispersion, in earlier research that were conducted by Bodmeier and Paeratakul (1994). These investigations

showed that the amount of the plasticizer partitioning was investigated.

Experimental Design Analysis. The utilisation of experimental design enables a methodical process of optimization, which involved the selection of an objective function, identification of most significant or influential causes, and examination of connection amongst responses along with factors through response surface methodology. Objective function chosen for this study was to maximise concentration of the polymer and the efficiency of the polymer coating, specifically the coat composition efficiency. The study aimed to investigate the impact of these factors on mucoadhesion, entrapment efficiency, in addition to drug release at the 9^{th} h, 12^{th} h, 18^{th} h and 24^{th} h for Azathioprine batches.

The Box-Behnken design was employed to statistically *optimize* processing parameters and evaluate impact of these parameters on concentration of polymer as well as enteric coating in the preparations. This design allowed for the evaluation of quadratic effects, interaction effects, besides key effects of processing parameters. Study employed a 3-factor, 3-level design to investigate quadratic response surfaces to develop second order polynomial models. This was accomplished utilizing Design Expert software (Version 9.0.5.1, Stat-Ease Inc., Minneapolis, MN). Box-Behnken design was chosen due to its ability to minimise the number of experimental runs required compared to a central composite design, particularly when dealing with three or four variables. The cubic design under consideration is distinguished by a collection of points situated at midpoint of every edge of a multidimensional cube, with the centre point being replicated once (n = 1). A design matrix comprising seventeen experimental runs was created to investigate linear computer-generated quadratic model for responses like entrapment efficiency (R1), mucoadhesion (R2), and drug release at 9^{th} h (R3), 12^{th} h (R4), 18^{th} h (R5), and 24^{th} h (R6) was given as:

$R1= 79.48 -0.0674 * A\ 2.31865 * B -0.10865 * C -3.065 * AB -0.16 * AC\ 0.1835 * BC\ 2.32135 * A^2 -13.75135 * B^2 -4.04115 * C^2$

$R2= 87.228 + 5.5 * A\ 2.44885 * B\ 0.65475 * C -3.18 * AB -0.93 * AC -0.3235 * BC -2.24765 * A^2 -2.75535 * B^2\ 0.98465 * C^2$

$R3= 70.178 + 5.64135 * A\ 2.07635 * B\ 1.2235 * C\ 0.3165 * AB -2.18 * AC -1.375 * BC\ 4.33965 * A^2 -1.86035 * B^2 -2.32765 * C^2$

$R4= 79.116 +4.0615 * A\ 2.7135 * B -1.235 * C\ 0.094 * AB\ 0.28 * AC -2.665 * BC\ 5.017 * A^2 -4.649 * B^2 -2.327 * C^2$

$R5= 83.62 + 3.9735 * A\ 0.88635 * B -0.06385 * C\ 0.067 * AB -0.265 * AC -0.0165 * BC\ 1.94135 * A^2 -0.81135 * B^2\ 0.95865 * C^2$

$R6= 87.614 + 1.90365 * A\ 0.51865 * B -0.18 * C -1.135 * AB -0.6135 * AC -0.1015 * BC\ 5.423 * A^2\ 0.394 * B^2\ 1.9456 * C^2$

The intercept for drug response R1 was determined to be 79.49, while the regression coefficients for the independent variables A, B, and C were calculated to be 2.31865, -0.10865, -3.065, -0.16, 0.1835, 2.32135, -13.75135 and -4.04115, respectively. These values were obtained from observed experimental values of R1 from the conducted experimental runs, where A, B, and C represent coded levels of the independent variables. Terms AB, AC, BC, A2, B2, and C2 denote interaction and quadratic terms, respectively. Aforementioned pattern was observed in the responses labelled as R2, R3, R4, R5, and R6.

The study focused on investigating the independent variable of polymer concentration, specifically Chitosan (A), Guar gum (B), as well as the coat composition of Eudragit L and S 100 (C). Entrapment efficiency (R1), mucoadhesion (R2), drug release at the 9th hour (R3), 12th hour (R4), 18th hour (R5), and 24th hour (R6) were considered as the dependent variables in this study. Range of independent variables being investigated was also included their low, medium, and high levels. These levels were determined grounded on findings obtained from preliminary experimentation. Seventeen experimental formulations were prepared using different polymer concentrations, namely Chitosan (A), Guar gum (B), and a coat composition of Eudragit L and S 100 (C). Polynomial equations can be utilised to derive inferences by taking into account magnitude and mathematical sign of coefficients. A value with a large +ve or -ve magnitude in equation specifies a slight adjustment to factor's setting can result in a substantial alteration in dependent variable.

The statistical validity of polynomials was determined through utilisation of the analysis of variance (ANOVA) feature provided by the Design Expert software. The level of significance was determined to be <0.05, as indicated by F statistic. Most suitable mathematical model was selected by evaluating numerous statistical parameters, such as coefficient of variation (CV), multiple correlation coefficient (R2), adjusted multiple correlation coefficient (adjusted R2), as well as predicted residual sum of squares (PRESS), as provided by software. The PRESS statistic is used to evaluate the goodness of fit of a model to data. In case of the selected model, it is desirable for the PRESS value to be relatively small compared to other models being considered. Design Expert® software was utilised to generate 3-D response surface graphs in addition to 2-D contour plots. The utilisation of these plots proved to be highly advantageous in visualising interaction effects of factors on responses.

In a previous study conducted by Shendge *et al* (2012), the authors investigated the statistical *optimiz*ation of Budesonide pellets coated with Eudragit polymer for potential colonic drug delivery. The researchers employed the Box-Behnken experimental design to *optimiz*e the processing parameters of the fluidized bed processor.

Full and Reduced Model assessment for the dependent variables. Outcome of the responses R1 to R6 yielded a success rate of 95% for the administration of Azathioprine. Responses obtained for seventeen processing variables were analysed and fitted to different models utilizing Design-Expert software. The analysis revealed that most suitable models for drug were Quadratic in nature. Table 3 presents the values of

R2, adjusted R2, predicted R2, standard deviation, and coefficient of variation, alongside regression equation derived for each response. The study revealed that independent variable, namely A (Chitosan), exhibited a favourable impact on the release of the drug (R). The additional independent variables, B (Guar gum) and C (Coat composition), were found to have a minimal impact on the outcome (R).

Table 3: Summary of outcomes of regression analysis for drug response R1 to R6.

Responses	Models	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	SD	%CV	PRESS
R1	Quadratic	0.9364	0.8522	-0.0321	8.5541	3.1097	4.3065	1080.75
R2	Quadratic	0.9295	0.8385	-0.1287	13.0012	2.0793	2.4371	482.96
R3	Quadratic	0.9041	0.7804	-0.5356	9.7747	2.5857	3.6803	747.67
R4	Quadratic	0.9817	0.9583	0.7116	22.7011	1.0816	1.3834	128.75
R5	Quadratic	0.9946	0.9878	0.9161	41.2670	0.3428	0.4048	13.11
R6	Quadratic	0.9573	0.9027	0.3462	12.0801	1.0842	1.1881	127.52

The entrapment efficiency was found to be negative in response R1. The term 'Pred R-Squared' suggests that the predictive power of the overall mean was superior to that of the current model in determining the response. The metric known as 'Adeq Precision' quantifies the ratio of signal to noise in a given system. A ratio exceeding 4 was considered favourable. The ratio of 8.5541 suggests a satisfactory signal. The aforementioned model possesses the capability to effectively navigate the design space. The presence of a negative 'Predicted R-Squared' indicates that the average value was a more effective predictor of the response compared to the existing model. The metric known as 'Adeq Precision' is utilised to quantify signal to noise ratio. A ratio exceeding 4 was considered favourable. The ratio of 13.0012 suggests a satisfactory signal. The aforementioned model possesses the capability to effectively navigate the design space. The negative value of the 'Pred R-Squared' metric suggests that the average value exhibited a higher level of effectiveness in predicting the response variable in comparison to the current model specifically during the 9th hour of drug release. The measurement of signal to noise ratio is conducted by 'Adeq Precision'. A ratio exceeding 4 was considered favourable. The ratio of 9.7747 suggests a satisfactory signal. The aforementioned model possesses the capability to effectively traverse and explore the various dimensions of the design space.

In regards to the drug release at the 12th hour, it is worth noting that 'Pred R-Squared' value of 0.7116 deviates significantly from 'Adj R-Squared' value of 0.9583. This discrepancy raises concerns about the accuracy and reliability of the model and/or data. Several factors that should be taken into account include model reduction, response transformation, and outliers. It is imperative to subject all empirical models to confirmation runs in order to ensure their validity and reliability. The metric known as 'Adeq Precision' quantifies signal to noise ratio. A ratio exceeding 4 was considered preferable. Ratio of 22.7011 suggests a satisfactory signal. The aforementioned model possesses the capability to effectively traverse the design space. In regards to the drug release at the 18th hour, it can be observed that 'Pred R-Squared' value of 0.9161 exhibited a satisfactory level of concordance with 'Adj R-Squared' value of 0.9878, as disparity between two values was below 0.2. The measurement of signal to noise ratio is conducted by 'Adeq Precision'. A ratio exceeding 4 was considered preferable. The ratio of 41.2670 suggests that the signal is sufficient. The aforementioned model possesses the capability to effectively traverse the design space. The discrepancy between the 'Pred R-Squared' value of 0.3462 and the 'Adj R-Squared' value of 0.9027 at the 24^{th} hour of drug release raises concerns about adequacy of model and/or quality of data. Several factors that should be taken into account include model reduction, response transformation, and outliers. Confirmation runs are necessary in order to test the validity of all empirical models. The metric known as 'Adeq Precision' quantifies the ratio of signal to noise. A ratio exceeding 4 was considered favourable. The ratio of 12.0801 suggests a satisfactory signal. The aforementioned model possesses the capability to effectively traverse the design space.

Polynomial equations produced by Design Expert were subjected to statistical validation, and the significance of models was estimated utilizing analysis of variance feature provided by software.

Evaluation for Multiparticulate:

Micromeritic studies

Micromeritic studies of trial batches of multiparticulate

Drug Azathioprine exhibits a range of average particle sizes in the trial batches, spanning from 1.1 ± 0.084 mm to 1.5 ± 0.012 mm. The particle size of multiparticulate systems exhibits variation depending on composition of polymer incorporated within formulation. Size range of multiparticulate is influenced by concentration of Chitosan, its molecular weight, and its viscosity. A reduction in concentration of Chitosan leads to a decrease in size of the multiparticulate. Measured tapped density values for the trial batches of azathioprine were found between 0.49 g/cm^3 to 0.55 g/cm^3, while bulk density values fell within range of 0.48-0.54 g/cm^3.

Table 4: Micromeritic studies of trial batches.

BatchCode	AverageParticle size(mm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle ofrepose (º)
C1a	1.1 ± 0.084	0.51 ± 0.02	0.55 ± 0.06	29.74±1.2
C2a	1.1 ± 0.014	0.49 ± 0.06	0.52 ± 0.04	28.54±1.1
C3a	1.3 ± 0.078	0.48 ± 0.03	0.50 ± 0.07	30.21±1.2
C4a	1.5 ± 0.012	0.52 ± 0.02	0.55 ± 0.04	29.84±1.3
C5a	1.4 ± 0.016	0.53 ± 0.04	0.51 ± 0.08	28.71±1.2
C1b	1.2 ± 0.014	0.52 ± 0.06	0.53 ± 0.04	29.74±1.2
C2b	1.4 ± 0.084	0.48 ± 0.04	0.49 ± 0.05	28.76±1.1
C3b	1.2 ± 0.014	0.54 ± 0.04	0.55 ± 0.06	31.21±1.2
C4b	1.2 ± 0.078	0.49 ± 0.02	0.51 ± 0.03	29.74±1.1
C5b	1.5 ± 0.012	0.48 ± 0.04	0.53 ± 0.07	28.54±1.2

* Each sample was analyzed in triplicate (n = 3)

Coat Composition a=10%, b=15%

Micromeritic studies of factorial batches 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 in the range of 0.48 to 0.62 g/cm^3. The angle of repose was determined to be within range of 25° to 35°, 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.

Swelling ratio of multiparticulate: The swelling ratio was determined in relation to the passage of time. The swelling ratio was observed to increase proportionally with rate of hydration and also increased as the multiparticulate rose in weight. When compared to other formulations, it was discovered that the swelling ratio of trial Batch C3a, C5a, C3b, and C5b was significantly higher. One possible explanation for this is because the formulation contains a higher concentration of chitosan. All of the batches expand gradually at first, but eventually reach their maximum size while maintaining their other concentrations, as indicated in the Table 6.

Table 5: Micromeritic studies of factorial batches of Azathioprine.

ParametersBatches	Average particle size(mm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle ofrepose (º)
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

*Each sample was analyzed in triplicate (n = 3)

Table 6: Swelling ratio of trial batches of Azathioprine.

BatchCode	Swelling ratio of multiparticulate adhering to the tissue In pH 7.4 Time/h							
	0	1	2	4	6	8	10	12
C1a	0	0.32 ±0.15	0.58±0.14	0.72±0.16	0.84±0.12	0.94±0.12	1.15±0.15	1.24±0.13
C2a	0	0.43±0.14	0.53±0.12	0.61±0.15	0.91±0.19	1.22±0.16	1.40±0.13	1.50±0.18
C3a	0	0.44±0.13	0.65±0.15	0.76±0.15	0.84±0.11	0.92±0.13	1.23±0.15	1.52±0.15
C4a	0	0.43±0.15	0.54±0.12	0.62±0.13	0.76±0.14	1.31±0.15	1.39±0.13	1.42±0.13
C5a	0	0.34±0.12	0.45±0.13	0.64±0.13	0.80±0.15	0.92±0.14	1.24±0.15	1.64±0.15
C1b	0	0.35±0.13	0.58±0.15	0.73±0.12	0.87±0.15	0.94±0.13	1.22±0.13	1.28±0.13
C2b	0	0.45±0.14	0.52±0.12	0.72±0.14	0.91±0.14	1.33±0.15	1.42±0.15	1.54±0.14
C3b	0	0.38±0.15	0.62±0.13	0.75±0.14	0.83±0.15	0.92±0.15	1.25±0.13	1.55±0.15
C4b	0	0.42±0.14	0.57±0.13	0.62±0.12	0.87±0.14	1.56±0.13	1.63±0.13	1.52±0.15
C5b	0	0.36±0.15	0.46±0.16	0.68±0.15	0.75±0.14	0.83±0.13	0.92±0.17	1.63±0.14

*Each sample was analyzed in triplicate (n = 3)

Swelling studies of factorial batches: 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 7.

Table 7: Swelling ratio of factorial batches of Azathioprine.

Batch Code	Swelling ratio of multiparticulate adhering to the 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

*Each sample was analyzed in triplicate (n = 3)

Percentage yield of multiparticulate of trial batches

Percentage yield of trial batches. A respectable percentage yield can be obtained from the prepared multiparticulate. After drying, weighing the powder results in determining the percentage yield of multiparticulate. According to the data presented in the Table 8, maximum % yield for the trial batches of azathioprine (C3a, C5a, C3b, and C5b) was determined to be 92.58 1.14, 95.32 1.45, 92.55 2.12, and 94.30 2.13%, correspondingly. It was originate that all batches of multiparticulate showed good percentage yield especially batches having more concentration of Chitosan gives excellent result; it may be due to increase viscosity of Chitosan as compared to Guar gum.

Table 8: Percentage yield of trial batches of Azathioprine.

Batch Code	% Yield
C1a	90.20± 1.22
C2a	91.83± 2.14
C3a	92.58± 1.14
C4a	91.01± 2.42
C5a	95.32± 1.45
C1b	90.16± 2.12
C2b	92.16± 1.15
C3b	92.55± 2.12
C4b	91.32± 2.42
C5b	94.30± 2.13

*Each sample was analyzed in triplicate (n = 3)

Percentage yield factorial batches: A 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 and molecular weight of polymer Chitosan and Guar gum as mentioned in table 9.

Table 9: 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

*Each sample was analyzed in triplicate (n = 3)

Drug entrapment study

Drug entrapment of trial batches. For the purpose of the drug entrapment investigation, the prepared multiparticulate was examined. The percentage of medicine that was discovered to be entrapped in all formulations was found to be satisfactory, which is defined as being greater than 67%. The percentage of drug that was found to be entrapped in experimental batches of azathioprine was found to be 72.37 1.13% for batch C3a, 84.68 1.15% for batch C5a, 79.31 1.15% for batch C3b, and 83.06 1.81% for batch C5b, respectively. The aforementioned formulations all demonstrated a higher entrapment efficiency, in contrast to other methods lower percentages of entrapment. This can be explained by polymer composition of chitosan and guar gum at a ratio of 2:1, which is displayed in Table 10.

Drug entrapment of factorial batches. Prepared A considerable percentage of the medicine is also entrapped in the factororial 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 increase in polymer concentration, particularly Chitosan, results in an increase in the amount of medicine that is entrapped, as shown in Table 11.

Table 10: % Drug entrapment of trial batches of Azathioprine.

Batch No.	Drug entrapment efficiency	
	Drug Concentration (mg)	% Drug entrapment
C1a	100	75.88± 1.41
C2a	100	68.12± 1.15
C3a	100	72.37± 1.13
C4a	100	74.56± 1.33
C5a	100	84.68± 1.15
C1b	100	74.68± 1.90
C2b	100	75.43± 1.62
C3b	100	79.31± 1.15
C4b	100	70.31± 1.40
C5b	100	83.06± 1.81

Each sample was analyzed in triplicate (n = 3)

Table 11: % Drug entrapped of factorial batches of Azathioprine.

Batch No.	Drug entrapment efficiency	
	Drug Concentration (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

*Each sample was analyzed in triplicate (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.

Table 12: % Cumulative release of trial batches of Azathioprine.

BatchCode	Time / h							
	0	**2**	**4**	**6**	**9**	**12**	**18**	**24**
C1a	0	20.15±2.52	29.18± 2.13	30.32± 2.31	76.97±2.28	81.29±3.53	84.04±2.23	85.61±2.71
C2a	0	19.01±2.33	23.14± 2.15	28.12±2.20	60.48±2.51	68.15±2.43	74.02±2.53	81.12±2.24
C3a	0	20.05±2.42	28.30± 2.23	30.12±3.04	64.53±2.32	77.68±3.62	80.62±3.46	82.70±2.52
C4a	0	20.55±2.16	26.38± 2.53	29.42±2.15	75.53±3.31	78.22±2.42	79.79±2.56	84.29±2.43
C5a	0	19.14±2.37	29.25± 3.46	30.27±2.11	84.19±2.41	89.68±3.24	91.53±2.26	92.87±2.41
C1b	0	20.09±3.52	24.18± 2.12	29.31±2.41	64.29±2.51	71.50±2.42	79.11±3.15	85.00±2.25
C2b	0	20.11±2.32	28.16± 3.44	30.30±3.21	67.35±2.15	76.25±3.42	80.21±2.53	82.89±2.33
C3b	0	20.21±2.47	28.51± 2.16	30.52±2.35	76.71±2.65	81.19±2.38	83.81±2.32	85.56±2.42
C4b	0	20.17±2.56	28.15± 3.43	30.42±2.70	75.17±3.43	80.13±2.27	84.13±2.43	86.22±2.51
C5b	0	20.13±2.55	25.17± 2.13	30.16±3.31	76.24±2.61	83.62±2.13	88.88±3.35	90.42±2.43

* Each sample was analyzed in triplicate (n = 3)

***Ex-vivo* mucoadhesion study of trial batches.** Through the use of an *ex-vivo* mucoadhesion study, the mucoadhesive characteristics of the multiparticulate were analysed. When the intervals of 12 hours had passed, weight of the multiparticulate that had leached out was determined.

C5a and C5b are the batches from the azathioprine study that demonstrate stronger adherence than the others. Their values are 81.03 1.30 and 79.48 1.37 respectively. Because of the rise in molecular weight of Chitosan and the higher viscosity of Chitosan in comparison to Guar gum, this result allows us to draw the conclusion that an increase in the concentration of polymer, particularly Chitosan, leads to an upsurge in adhesion of multiparticulate. This data also confirms that there was no influence of coat composition on adherence, as both batches show the same outcome, regardless of whether the coat content is 10% (low) or 15% (high), as indicated in the Table 13. This is the case because both batches exhibit the same result.

***Ex-vivo* mucoadhesion study of factorial batches of Azathioprine.** Additionally, factorial batches have good adherence, which is defined as greater than 68%. Regarding the azathioprine factorial batches, the adhesion of batch A4 (90.86 2.02%) is superior to that of the other batches. Based on the results of these factorial experiments, we may draw the conclusion that an upsurge in polymer concentration results in an improvement in adhesion, and that adhesion was not effected by coat composition, as shown in Table 14.

Table 13: *Ex-vivo* mucoadhesion study of trial batches of Azathioprine.

Batch Code	Wt. of applied multiparticulate (mg)	Wt. of leached multiparticulate after 12 h (mg)	% Mucoadhesion
C1a	630± 0.42	130± 0.12	79.36± 1.11
C2a	600± 0.32	158± 0.15	73.66± 1.42
C3a	625± 0.21	147± 0.22	76.48± 1.35
C4a	610± 0.42	150± 0.32	75.40± 1.36
C5a	580± 0.50	110± 0.36	81.03± 1.30
C1b	630± 0.34	130± 0.22	79.36± 1.34
C2b	600± 0.28	135± 0.42	77.50± 1.31
C3b	575± 0.36	125± 0.30	78.26± 1.22
C4b	600± 0.31	145± 0.22	75.83± 1.25
C5b	585± 0.30	120± 0.62	79.48± 1.37

* Each sample was analyzed in triplicate (n = 3)

Table 14: *Ex-vivo* mucoadhesion study of factorial batches of Azathioprine.

Batch Code	Wt. of applied multiparticulate (mg)	Wt. of leached multiparticulate after 12 h (mg)	% Mucoadhesion
C1	600± 0.42	78± 0.12	87.00± 1.12
C2	600± 0.32	65± 0.15	89.16± 1.06
C3	625± 0.21	87± 0.22	86.08± 1.15
C4	580± 0.50	53± 0.36	90.86± 2.02
C5	580± 0.50	63± 0.36	89.13± 1.12
C6	590± 0.34	65± 0.22	89.83± 1.03
C7	600± 0.28	80± 0.42	86.66± 1.02
C8	575± 0.36	84± 0.30	85.39± 1.2
C9	600± 0.31	80± 0.22	86.66± 0.12
C10	585± 0.30	65± 0.62	88.88± 1.03
C11	580± 0.50	88± 0.36	84.82± 1.12
C12	600± 0.34	85± 0.22	85.83± 1.06
C13	600± 0.28	80± 0.42	86.66± 0.32
C14	575± 0.36	83± 0.30	85.56± 0.1
C15	600± 0.31	88± 0.22	85.33± 0.13
C16	585± 0.30	195± 0.62	66.66± 0.12
C17	580± 0.50	120± 0.36	79.31± 0.14

* Each sample was analyzed in triplicate (n = 3)

***In-vitro* drug release studies for factorial batches:** 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.

Observing prolonged release characteristics of coated multiparticulate in GIT was an intriguing experience. It is possible that this is because of cross-linking of chitosan, which did not disintegrate when subjected to action of colon enzymes. As a result, it is possible to assert that the multiparticulate maintained their integrity. It's possible that the release will happen either by diffusion or erosion. However, it was seen that around 30% of medication got release from multiparticulate and around 76 to 86 % drug release after 9 h for azathioprine. This may be due to dissolution of Eudragit S&L 100 in alkaline fluid.

Statistical analysis. After determining slope of the appropriate equations, correlation coefficient (R) was then calculated for each of formulations (Tien Bui *et al.*, 2019). This allowed release rate constant to be determined. When compared to other formulations, it was discovered that release profile and 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 the release of the medication closely followed the Higuchi kinetics, which suggests that drug 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 and guar gum, the results of our study unequivocally support the non-fickian model of diffusion. The findings are presented in the Table 16.

Table 15: % 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

*Each sample was analyzed in triplicate (n = 3)

Table 16: Kinetic parameters of azathioprine release from factorialbatches of multiparticulate.

Batch Code	Zero-order		First-order		Higuchi		Hixson-Crowell		k-Peppas		
	K_0	R^2	K_1	R^2	K_H	R^2	K_{HC}	R^2	Kp	R^2	np
C1	3.9448	0.8424	0.1272	0.9773	21.8207	0.9376	-0.1254	0.9498	1.0985	0.9371	0.8868
C2	4.0195	0.8225	0.1365	0.9673	22.3433	0.9245	-0.1316	0.9302	1.1035	0.9279	0.8973
C3	3.7218	0.8304	0.0995	0.9512	20.7428	0.9382	-0.1070	0.9169	1.1035	0.9379	0.8659
C4	4.1014	0.8222	0.0996	0.9811	22.7761	0.9944	-0.1423	0.9420	1.0808	0.9979	0.8054
C5	3.6626	0.8239	0.0940	0.9380	20.4568	0.9349	-0.1029	0.9046	1.0984	0.9344	0.8742
C6	3.6634	0.8219	0.0933	0.9310	20.4472	0.9314	-0.1025	0.8987	1.0939	0.9325	0.8697
C7	4.0293	0.8116	0.1364	0.9584	22.4602	0.9173	-0.1318	0.9164	1.0999	0.9230	0.9233
C8	3.8046	0.8643	0.1112	0.9776	20.957	0.9539	-0.1145	0.9610	1.0828	0.9508	0.8816
C9	3.6676	0.8231	0.0942	0.9360	20.4850	0.9340	-0.1031	0.9029	1.0961	0.9342	0.8778
C10	3.6379	0.8175	0.0923	0.9275	20.3689	0.9323	-0.1016	0.8951	1.1044	0.9320	0.8704
C11	3.8539	0.8602	0.1136	0.9691	21.1881	0.9454	-0.1164	0.9533	1.0561	0.9464	0.9088
C12	3.7254	0.8894	0.0980	0.9733	20.3712	0.9674	-0.1069	0.9756	1.0621	0.9647	0.8628
C13	3.7088	0.8665	0.0978	0.9803	20.4269	0.9561	-0.1057	0.9542	1.0688	0.9526	0.8988
C14	3.6490	0.8201	0.0928	0.9297	20.4024	0.9325	-0.1020	0.8973	1.0993	0.9339	0.8729
C15	3.8331	0.8889	0.11003	0.9223	20.9073	0.9620	-0.1142	0.9711	1.0504	0.9652	0.8434
C16	3.6213	0.8502	0.0916	0.9676	20.0525	0.9483	-0.1007	0.9375	1.0822	0.9440	0.8732
C17	3.6878	0.9016	0.1571	0.9727	20.1180	0.9760	-0.1055	0.9834	1.1048	0.9265	0.9041

CONCLUSIONS

Colon drug delivery system of Azathioprine was successfully prepared and 2^3 factorial design model was employed to optimize the formulation. 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. Then the optimized formulation C4 is suggested for further evaluation.

FUTURE SCOPE

A novel technique for treating intestinal illnesses is the oral colon delivery system. The optimized colon drug delivery system of Azathioprine could be used for further evaluation for clinical application.

Acknowledgment. The authors deeply appreciate the assistance of the Department of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India.
Conflict of Interest. None.

REFERENCES

Akl, M. A., Kartal-Hodzic, A., Oksanen, T., Ismael, H. R., Afouna, M. M., Yliperttula, M., Samy, A.M. & Viitala, T. (2016). Factorial design formulation optimization and in vitro characterization of curcumin-loaded PLGA nanoparticles for colon delivery. *Journal of Drug Delivery Science and Technology*, *32*, 10-20.

Belgamwar, V., Shah, V., & Surana, S. J. (2009). Formulation and evaluation of oral mucoadhesive multiparticulate system containing metoprolol tartarate: an in vitro-ex vivo characterization. *Current drug delivery*, *6*(1), 113-121.

Bourgeois, S., Harvey, R., & Fattal, E. (2005). Polymer colon drug delivery systems and their application to peptides, proteins, and nucleic acids. *American journal of drug delivery*, *3*, 171-204.

Broen, J. C., & van Laar, J. M. (2020). Mycophenolate mofetil, azathioprine and tacrolimus: mechanisms in rheumatology. *Nature Reviews Rheumatology*, *16*(3), 167-178.

Bruno, B. J., Miller, G. D., & Lim, C. S. (2013). Basics and recent advances in peptide and protein drug delivery. *Therapeutic delivery*, *4*(11), 1443-1467.

Chen, Y., Yang, H., Zou, H., Sun, T., Li, M., Zhai, J., He, Q., Gu, L., & Tang, W. Z. (2020). Effects of acid/alkali pretreatments on lignocellulosic biomass mono-digestion and its co-digestion with waste activated sludge. *Journal of Cleaner Production*, *277*, 123998.

de Sousa Victor, R., Marcelo da Cunha Santos, A., Viana de Sousa, B., de Araújo Neves, G., Navarro de Lima Santana, L., & Rodrigues Menezes, R. (2020). A review on Chitosan's uses as biomaterial: Tissue engineering, drug delivery systems and cancer treatment. *Materials*, *13*(21), 4995.

Dhole, A. R., Gaikwad, P. D., Bankar, V. H., & Pawar, S. P. (2011). A review on floating multiparticulate drug delivery system-A novel approach to gastric retention. *Int J Pharm Sci Rev Res*, *2*, 205-11.

Elnaggar, Y. S., El-Massik, M. A., & Abdallah, O. Y. (2009). Self-nanoemulsifying drug delivery systems of tamoxifen citrate: design and optimization. *International journal of pharmaceutics*, *380*(1-2), 133-141.

Farasati Far, B., Naimi-Jamal, M. R., Safaei, M., Zarei, K., Moradi, M., & Yazdani Nezhad, H. (2022). A Review on biomedical application of polysaccharide-based hydrogels with a focus on drug delivery systems. *Polymers*, *14*(24), 5432.

Florek, J., Caillard, R., & Kleitz, F. (2017). Evaluation of mesoporous silica nanoparticles for oral drug delivery–current status and perspective of MSNs drug carriers. *Nanoscale*, *9*(40), 15252-15277.

Ghosh, S., Ghosh, S., & Sil, P. C. (2019). Role of nanostructures in improvising oral medicine. *Toxicology Reports*, *6*, 358-368.

Kumar, D. V. N, Narasimha Ra, C., Srineeth, U., Nageswara Redd, C., Sachidevi, P., and Prakash Rao, S. (2023). Significant Impact of Ginger Extract on Oxidative stress Markers and Lipid Peroxidation in Diabetic Male Albino Rats. *Biological Forum – An International Journal*, *15*(1), 412-418.

Lorscheider, M., Gaudin, A., Nakhlé, J., Veiman, K. L., Richard, J., & Chassaing, C. (2021). Challenges and opportunities in the delivery of cancer therapeutics: update on recent progress. *Therapeutic Delivery*, *12*(1), 55-76.

Masaki, Y., Nakase, H., Tsuji, Y., Nojima, M., Shimizu, K., Mizuno, N., Ikeura, T., Uchida, K., Ido, A., Kodama, Y., & Masamune, A. (2021). The clinical efficacy of azathioprine as maintenance treatment for autoimmune pancreatitis: a systematic review and meta-analysis. *Journal of gastroenterology*, *56*, 869-880.

Naeem, M., Choi, M., Cao, J., Lee, Y., Ikram, M., Yoon, S., Lee, J., Moon, H.R., Kim, M.S., Jung, Y. & Yoo, J. W. (2015). Colon-targeted delivery of budesonide using dual pH-and time-dependent polymeric nanoparticles for colitis therapy. *Drug design, development and therapy*, 3789-3799.

Pandiya, H., & Sharma, C. S. (2021). A Review on Gastroretentive Drug Delivery System of Antihypertensive Drugs. *The Pharmaceutical and Chemical Journal*, *8*(3), 51-76.

Patel, A. A., Swerlick, R. A., & McCall, C. O. (2006). Azathioprine in dermatology: the past, the present, and the future. *Journal of the American Academy of Dermatology*, *55*(3), 369-389.

Rajpurohit, H., Sharma, P., Sharma, S., & Bhandari, A. (2010). Polymers for colon targeted drug delivery. *Indian journal of pharmaceutical sciences*, *72*(6), 689.

Remmele, R. L., Nightlinger, N. S., Srinivasan, S., & Gombotz, W. R. (1998). Interleukin-1 receptor (IL-1R) liquid formulation development using differential scanning calorimetry. *Pharmaceutical research*, *15*, 200-208.

Sagar, R.K., Kulkarni, M.P., Vandana P.B., Wadhwa, S., Singh, G., and Sharma, A. (2021). Minitablets: Recent Trends and Developments with an update on Research and Patents. *Biological Forum – An International Journal*, *13*(2), 01-09.

Shekunov, B. Y., Chattopadhyay, P., Tong, H. H., & Chow, A. H. (2007). Particle size analysis in pharmaceutics: principles, methods and applications. *Pharmaceutical research*, *24*, 203-227.

Sivamaruthi, B. S., kumar Nallasamy, P., Suganthy, N., Kesika, P., & Chaiyasut, C. (2022). Pharmaceutical and biomedical applications of starch-based drug delivery system: A review. *Journal of Drug Delivery Science and Technology*, 103890.

Taniguchi, C., Kawabata, Y., Wada, K., Yamada, S., & Onoue, S. (2014). Microenvironmental pH-modification to improve dissolution behavior and oral absorption for drugs with pH-dependent solubility. *Expert opinion on drug delivery*, *11*(4), 505-516.

Tien Bui, D., Moayedi, H., Gör, M., Jaafari, A., & Foong, L. K. (2019). Predicting slope stability failure through machine learning paradigms. *ISPRS International Journal of Geo-Information*, *8*(9), 395.

Vass, P., Démuth, B., Hirsch, E., Nagy, B., Andersen, S. K., Vigh, T., Verreck, G., Csontos, I., Nagy, Z.K. & Marosi, G. (2019). Drying technology strategies for colon-targeted oral delivery of biopharmaceuticals. *Journal of controlled release*, *296*, 162-178.

Zhang, Z., He, S., Liu, H., Sun, X., Ye, Y., Cao, X., Wu, Z., & Sun, H. (2020). Effect of pH regulation on the components and functional properties of proteins isolated from cold-pressed rapeseed meal through alkaline extraction and acid precipitation. *Food chemistry*, *327*, 126998.

How to cite this article: Shikha Jakhotiya and Gajendra Singh Rathore (2023). Factorial Design Formulation Optimization and *in vitro* characterization of Colon Targeted Delivery System of Azathioprine. *Biological Forum – An International Journal, 15*(5a): 492-505.