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Research Paper

Formulation And Evaluation of Colon Targeted Delivery of Mesalamine for The Treatment of Ulcerative Colitis

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ABSTRACT
Conventional oral medications often fail to effectively treat colonic diseases, but Mesalamine is a preferred treatment for ulcerative colitis. This study prepared and optimized mesalamine microspheres using solvent evaporation method using cellulose acetate phthalate as enteric coating polymer for colon targeting. Box-Behnken design using design expert software was employed in formulating and optimizing the microspheres with three independent variables that is polymer concentration (X_1), stirring speed (X_2), and surfactant concentration (X_3) and four dependent variables such as particle size, percentage entrapment efficiency, invitro drug release. The prepared microspheres underwent various evaluations, including particle size, drug entrapment, and release studies. Additionally, Fourier Transformed -Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM) studies analyses confirmed drug-excipient compatibility and revealed the microspheres spherical shape. Optimized mesalamine microspheres (F7) showed high drug entrapment efficiency (87.55%), controlled release (1.06% in acidic medium, 93.59% at pH 6.8), and super case II transport mechanism, making them a promising carrier for colon-targeted drug delivery.

Keywords: *Mesalamine, Ulcerative colitis, Cellulose acetate phthalate, Box Behnken design*

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I. INTRODUCTION

The oral route is considered to be the most preferred route for administration of drugs for systemic effect, but the oral route is not suitable to the administration of drug for lower gastrointestinal (GI) diseases, this happened due to their release at upper GI tract (stomach, small intestine), which further minimizes the accessibility of drugs at the lower GI tract. To overcome this difficulty, colon-specific drug delivery systems have been broadly analysed during the last two decades. [1,2]

Colon specific diseases are often inefficiently managed by oral therapy, because most orally administered drugs are absorbed before arriving in the colon. Therefore, colon specific drug delivery systems, which can deliver drugs to the lower gastrointestinal tract without releasing them in the upper GI-tract, can be expected to increase the quality of life for patients suffering from colon specific diseases [3].

Treatment might be more effective if the drug substances were targeted directly on the site of action in the colon. Lower doses might be adequate and, if so, systemic side effects might be reduced. Colon specific drug delivery systems have gained increasing attention for the treatment of diseases such as Crohn's disease, ulcerative colitis and irritable bowel syndrome. There are several approaches, which are utilized in achieving

colon targeting, among all microparticulate drug delivery is regarded as the better method for the controlled delivery of drug into the specific sites of inflammation. [4]

Microspheres constitute an important part of this particulate drug delivery system by virtue of their small size and efficient carrier characteristics. However, the success of this novel drug delivery system is limited due to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with absorbing gastric mucosal membranes. Microspheres are characteristically free powders consisting of proteins or synthetic polymers that are biodegradable in nature and ideally having a particle size less than 200µm.

Ulcerative colitis is a type of inflammatory bowel disease (IBD) that affects the lining of the large intestine (colon) and rectum. Repeated swelling (inflammation) leads to thickening of the intestinal wall and rectum with scar tissue. Death of colon tissue or severe infection (sepsis) may occur with severe disease.

Mesalamine is an anti-inflammatory drug used to treat Crohn's disease and ulcerative colitis. Since Mesalamine is largely absorbed from the upper intestine, selective delivery of drugs into the colon may be regarded as a better method of drug delivery with fewer side effects and a higher efficacy. [4,5]

II. MATERIALS AND METHODS

Materials

Mesalamine was a gift sample from Tablets (India) Ltd, Chennai. Cellulose acetate phthalate, span 80, Dichloromethane, Liquid paraffin was purchased from We associates, Kottayam. All other chemicals used in experiment were of analytical grade and used as such.

Methods

Preformulation Studies:

Preformulation studies are crucial in drug development, investigating a drug's physical and chemical properties with and without excipients. This research focuses on preformulation studies for Mesalamine, aiming to develop a colon-targeted delivery system for ulcerative colitis. Key parameters assessed include melting point, solubility, particle size, and compatibility with excipients.

Physical Characterization of Drug Sample:

Nature: The drug sample's physical nature was assessed visually and with a compound microscope.

Colour: The colour of the drug sample was observed visually against contrasting backgrounds.

Melting Point: The melting point of drug was determined by capillary tube method. The drug was filled to capillary tube which has one end sealed. The filled capillary tube was placed inside the melting point apparatus and the temperature at which drug melted was noted.

Solubility: A solubility test was conducted by accurately weighing 100mg of the drug and transferring it into a stoppered tube containing 0.1ml of solvent. If the drug completely dissolved, it was classified as very soluble. If not, an additional 0.9ml of solvent was added, and if dissolution occurred, the drug was considered freely soluble. Further increments of solvent were added in steps, with the drug being classified as soluble, sparingly soluble, or slightly soluble based on its dissolution at each step. If the drug remained undissolved, a smaller amount of 1mg was tested with 10ml of solvent, and if dissolution occurred, it was classified as very slightly soluble.

Calibration curve for Mesalamine

UV spectrophotometry method was developed for the analysis of drug using double beam Systronics-2202 spectrophotometer.

Determination of λmax

Mesalamine at a concentration of (100µg/ml) was dissolved in 0.N HCl and scanned for maximum absorbance in UV double beam spectrophotometer in the range from 200 to 400 nm against buffer as blank.

Preparation of Standard Stock Solution

100 mg of Mesalamine was accurately weighed and transferred into 100 ml volumetric flask. The drug was dissolved and diluted to volume with 0.1N HCL to get concentration of 1000µg/ml.

Preparation of Working Standard Solution

10 ml of the stock solution was pipetted out from standard stock solution and diluted to 100ml with 0.1N HCL to get a concentration of 100µg/ml.

Standard Calibration Curve for Mesalamine

Series of solutions with concentration range of 2, 4, 6, 8, 10, 12, 14, 16, 18, 20µg/ml were prepared by pipetting 2, 4, 6, 8, 10, 12, 14, 16, 18, 20ml from stock solution and made up to 100ml with 0.1N HCL. The absorbance of these solutions was measured at 230 nm by UV spectrophotometer, using 0.1N HCL as blank. The calibration curve was prepared by plotting absorbance against concentration.

Drug excipients Compatibility Studies:

FTIR (Fourier Transform Infrared) Studies:
The IR spectra were recorded using FTIR spectrophotometer. The samples were prepared by mixing the drug and the excipients in 1:1 ratio and the mixtures were stored in closed containers for 1 week. FTIR spectrum of the samples was taken using potassium bromide pellet method. The physical mixtures of Betaxolol HCl and excipients were scanned in the wavelength region between 3800 and 650 cm-1 and compared to check compatibility of drug with excipient.

DSC (Differential Scanning Calorimetric) Analysis:
DSC study was carried out using DSC-60 instrument to check the compatibility of ingredients. The samples were prepared by mixing the drug and the excipients in 1:1 ratio. Accurately weighed samples were sealed in aluminium pans and analysed in an inert atmosphere of nitrogen at flow rate of 25 ml/min. A temperature range of 0°C to 300°C was used, and the heating rate was 10°C/min. DSC thermograms of pure drugs and physical mixtures of drugs and excipients were studied for their interactions.

Optimization employing Box Behnken design
To design colon-specific microspheres, key formulation parameters were identified and optimized using the Box-Behnken statistical design. This efficient method was employed to optimize mesalamine microspheres for colon-targeted drug delivery using Design Expert Software.
To optimize a formulation, three independent variables - polymer concentration (X_1), stirring speed (X_2), and surfactant concentration (X_3) - were studied at three levels each. The effects of these variables on four response factors - particle size (Y_1), % drug entrapmentefficiency (Y_2), % drug release in 3 hrs (Y_3) and % drug release in 12 hours (Y_4) - were evaluated. Statistical analysis was performed using various parameters, including p-value, R2 value, F value, and lack of fit F value, to develop a mathematical model that best fit the data. Quadratic polynomial response equations were also developed to understand the interactions between key factors. The validity of the model was assessed using ANOVA, and the optimal formulation was selected based on the response variables. The polynomial equation generated by the experimental design is as follows:
$y = \beta0 + \beta1X1 + \beta2X2 + \beta3X3 + \beta4X1X2 + \beta5X2X3 + \beta6X1X3 + \beta7X12 + \beta8X22 + \beta9X32\ldots.$
Here, y represents the measured response, β0– β9 are regression coefficients and X1, X2, and X3 are independent factors. By applying analysis of variance (ANOVA), lack of fit, and coefficient of determination (R2) as a measure of goodness of fit of the fitted model, models were validated.

Table 1: Independent and dependent variables

Independent variable	**Variable level**		
	Low (-1)	**Medium (0)**	**High (+1)**
X_1 = Polymer concentration	400	800	1200
X_2 = Stirring speed	1500	2000	2500
X_3 = Surfactant concentration	0.5	1.0	1.5
Dependent variable (Response)			
Y_1 = Particle size (μm)			
Y_2= Percent Entrapment efficiency (%EE)			
Y_3= Drug release in 3 hrs (%)			
Y_4 = Drug release in 12 hrs (%)			

Table 2: Box Behnken Design layout for optimization of mesalamine microspheres

Formulation Code	Run order	X_1-Polymer conc. (mg)	X_2–Stirring speed (rpm)	X_3–Surfactant conc. (%)
F1	1	1200	2000	1.5
F2	2	800	2500	1.5
F3	3	400	2500	1
F4	4	800	2000	1
F5	5	1200	2000	0.5
F6	6	1200	1500	1
F7	7	800	2000	1
F8	8	800	2500	0.5
F9	9	800	1500	1.5
F10	10	1200	2500	1
F11	11	400	2000	0.5
F12	12	800	2000	1
F13	13	400	2000	1.5
F14	14	800	2000	0.5
F15	15	800	2000	1
F16	16	800	2000	1
F17	17	400	1500	1

Formulation of Microspheres

Microspheres were prepared by solvent evaporation method. Accurately weighed quantity of the polymer was dissolved in dimethyl formamide. Weighed quantity of mesalamine (drug) was then dispersed in the above polymer solution. The resulting suspension was added dropwise to the liquid paraffin containing span 80 and stirred for 2 hrs using a propeller stirrer. Stirring was continued until the organic solvent evaporated completely. Then the obtained microspheres were filtered, washed with petroleum ether, dried overnight and then stored in desiccators until further use.

Table 3: Formulation of microspheres

Formulation code	Ingredients			
	Mesalamine (mg)	Cellulose acetate phthalate (mg)	Liquid paraffin (ml)	Span 80 (%)
F1	400	1200	100	1.5
F2	400	800	100	1.5
F3	400	400	100	1
F4	400	800	100	1
F5	400	1200	100	0.5
F6	400	1200	100	1
F7	400	800	100	1
F8	400	800	100	0.5
F9	400	800	100	1.5
F10	400	1200	100	1
F11	400	400	100	0.5
F12	400	800	100	1
F13	400	400	100	1.5
F14	400	800	100	0.5
F15	400	800	100	1
F16	400	800	100	1
F17	400	400	100	1

Evaluation of Microspheres
Micromeritic Properties

AngleofRepose
Angle of repose was determined by measuring the height, radius of the heap of the powder blend. A cut system funnel was fixed to a stand and bottom of the funnel was fixed at a height of 2 cm from the plane. Powder blend was placed in funnel and allowed to flow freelyand measured the height and radius of the heap.
Tan(θ)= h/r
Where,h =height ofheap, r =radiusof heap
Bulk density
Bulk density (Db) is the ratio of total mass of powder to the bulk volume of powder. Accurately weighed quantities of the blended mixture (10gm) were carefully poured into the graduated cylinder through a funnel and the bulk volume was recorded with and without tapping. Bulk density was calculated by taking the ratio of mass of powder and bulk volume of powder.
Itisexpresseding/mlandisgivenby,
Db = M /Vo
M -Themass ofpowder, Vo-Thebulkvolumeofpowder(ml)
Tappeddensity
Tapped density is the bulk density of a powder which has been compacted by tapping or vibration.Tappeddensitywasdeterminedbyplacinga graduatedcylinder containingaknownmass of powder on a mechanical tapping apparatus, which is operated for a fixed number of taps (100) or until the powder bed volume has reached a minimum. The tapped density was computed by taking the weight of drug in cylinder and final volume.
Tappeddensity=Weightofpowder/tappedvolume
Compressibilityindex(Carr'sindex)
Another indirect method of measuring powder flow form bulk densities was developed by Carr. The percentage compressibility of a powder is a direct measure of the potential powder archor bridge strength and stability. It is calculated according to the following equation,
Carr'sindex=Tappeddensity-bulkdensity/Tappeddensity×100
Hausner'sratio
Hausner ratio is an indirect index of ease of powder flow. If the hausner's ratio of powder is near to 1.25, indicates better powder flow. It is calculated by the following formula,
Hausner'ratio=Tappeddensity/Bulkdensity
Particlesize determination
Particle size of the microspheres was evaluated using optical microscopy method. Approximately 100 microspheres were counted for particle size determination using a calibrated optical microscope. The experiments were performed in triplicate (n=3).
Percentageyield ofMicrospheres
The prepared microspheres of all batches were accurately weighed. The weight quantity of prepared microspheres was divided by the total amount of all the excipients and drug used in the preparation of the microspheres, which give the total percentage yield of microspheres. It was calculated by using following formula.
Percentageyield=(Practicalyield/Theoretical yield)×100
Shapeandsurfacemorphology
The shape and surface morphology of Mesalamine microspheres were investigated using scanning electron microscopy. The coated samples were then randomly scanned and photomicrographs were taken with the help of scanning electron microscope.
Entrapmentefficiency
Accordingtotheactualdrugpresentin totalmicrospheresis calculated.That amountmixed in0.1 N HCL by sonication. From that pipette 1ml into 10ml volumetric flask and made up to 10ml using buffer. Thesolution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 230nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula.
Percentagedrugentrapment=Actualdrugcontent/Theoreticaldrug contentX100
***Invitro*drug release**
Dissolution was performed using USPtype I (basket) apparatus. The drug loaded microspheres equivalent to 100 mg of mesalamine were introduced into 900 ml of dissolution medium which was maintained at 37±0.5ºC and stirred at 100rpm. The dissolution was carried out in 0.1N hydrochloric acid for the first 3hrs followed by 6.8 for the next 21 hrs to mimic the GIT transit to colon region. Aliquots sample (5ml) was withdrawn from the dissolution apparatus at the appropriate time intervals and sink conditions were maintained. Absorbance of the samples was measured at 230nm for Mesalamine using UV-Visible double-beam spectrophotometer. The drug

content was calculated using the equation generated from standard calibration curve. The cumulative % drug release was calculated.

RELEASEKINETICS

Kinetic study was carried out by fitting the in vitro drug release data into Zero order, First order, Higuchi model, Hixson-Crowell Cube Root Law model and Korsmeyer-Peppas models. The best fit model was confirmed by the value of R_2which is near to 1.

ZeroOrderKinetics

Plotmadebetweencumulative% drugreleasevs.time. Mathematicalrelationshowsthat the releaseisindependentof drug concentration.

$$Q=Q_0+k_0t$$

Where, **Q** is the amount of drug released or dissolved; Q_0 is the initial amount of drug in the solution (most times, Q0 =0); k_0 is the zero order release constant expressed in units of concentration/time.

FirstOrderKinetics

Plot made between log cumulative % drug retained vs. time would yield a straight line with a slope of -k/2.303. Mathematical relation shows that the release is proportional to amount of drug remaining.

$$\log C= \log C_0 - kt/2.303$$

Where,C_0 istheinitialconcentrationofdrug;**k**isthefirstorder rate constant;**t**isthe time.

HiguchiModel

Plot made between cumulative % drug release vs. √t. Mathematical relation shows that the releaseis proportional to square root of time.

$$Q_t=K_Ht^{1/2}$$

Where, Q_t is the amount of drug released at time **'t'**; K_H is the release rate constant for the Higuchi model.

HixsonCrowellModel

A plot of cube root of % cumulative drug remaining in matrix vs. time was made. This mathematical model describes drug release as dissolution from erodible matrix formulations. Here, the relation shows that the particles regular area is proportional to the cube root of its volume.

$$W_0^{1/3}-W_t^{1/3}=ṭt.$$

Where, W_0 is the initial amount of drug in pharmaceutical dosage form; W_t is the remaining amount of drug in the pharmaceutical dosage form at time **'t'** and **ṭ** (kappa) is a constant incorporating the surface volume relation.

Korsmeyer-PeppasModel

Plot made between log of cumulative % drug release vs. log time. The Korsmeyer-Peppas power law equation predicts that the fraction release of drug is exponentially related to the release time and adequately describes the release of drug from slabs, cylinders and spheres. This was used to find out the mechanism of drug release.

$$M_t/M_\infty=k_tn$$

Where, M_t/ M_∞ is a fraction of drug released at time't'; k is the release rate constant; **n** is the release exponent. The **'n'** value is used to characterize different release mechanism of drug. If n is less than 0.5,then thesystem follows fickian diffusion mechanism, if n value is greater than 0.5 and lessthan1.0,thenthedrugtransportmechanismfollowsnon-fickianoranomalousdiffusion.If release exponent is more than 1, the system follows case II transport.

Table 4: Diffusion Exponent and Solute Release Mechanism

Diffusionexponent(n)	Overallsolutediffusion mechanism
0.45	Fickiandiffusion
0.45<n<0.89	Anomalous(non-Fickian)diffusion
0.89	Case-IItransport
n<0.89	Supercase-IItransport

STABILITY STUDIES

The stability studies were carried out as per ICH guidelines. The optimized formulation was subjected to accelerated stabilitystudies for a period of 6 months at a temperature of 40ºC±2°C and Relative Humidity (RH) 75%RH±5% RH in a stability chamber. Samples were withdrawn at an intervaloftimeandanalysedsuitablyforEntrapmentefficiencyanddissolutioncharacteristics.

III. RESULTS AND DISCUSSION

Preformulation study

Identification of Drug

The sample spectrum was compared with the reference spectra and there were no significant changes in the functional groups. The frequency of observed functional groups C=C, C=O, C-O, and O-H are within the standard limits [Table No.5]. The fingerprint area has no change. So, the drug was identified as Mesalamine.

Organoleptic Evaluation

- Color: Almost white or light grey or light pink
- Odour: Slight characteristic odour
- Appearance: Crystalline powder

Determination of Melting Point

The standard melting point in the range of 281-283°C. The observed value was 283°C and was within the range as per the official standard.

Sl. No.	Drug	Functional Groups (cm^{-1})				
		C-O	C-H	C=C	C=O	O-H
1.	Reference Mesalamine	1242	1431	1582	1654	2772
2.	Sample Mesalamine	1235	1444	1572	1644	2769

Figure 1: Reference spectrum of Mesalamine

Table 5: Functional Group and their Observed Peak Values

CONCENTRATION (µg/ml)		ABSORBANCE
0		0
2		0.228
4		0.459
6		0.594
8		0.785
10		0.995

Solubility Study

Table 6: Solubility of Mesalamine

Sl.No	Solvent	Solubility
1	Water	Slightly soluble
2	Methanol	Very slightly soluble
3	Acetone	Very slightly soluble
4	0.1N HCl	Soluble
5	Dil. alkali hydroxides	Soluble
6	Alcohol	Insoluble

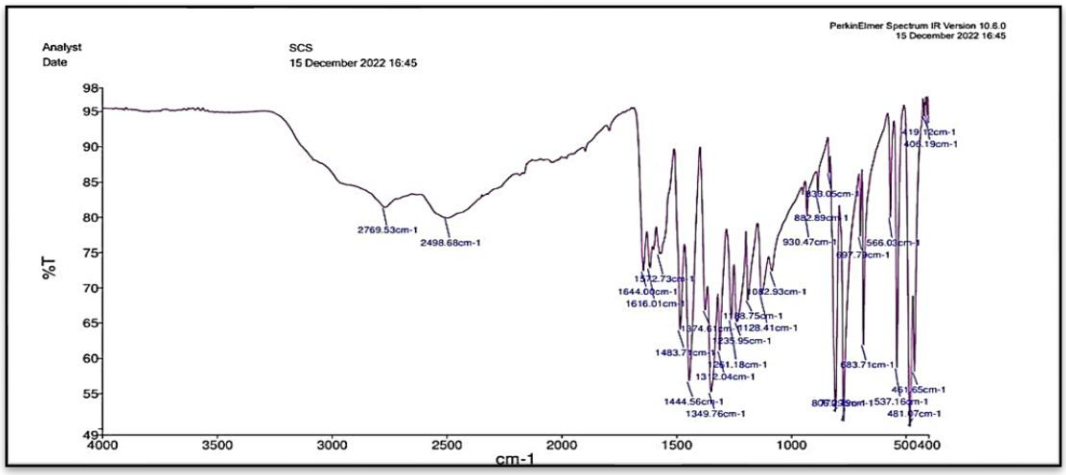

Figure 2: IR spectrum of Mesalamine (sample)

The solubility was determined by dissolving the drug in various solvents like water, methanol, acetone 0.1N HCl, dil.alkali hydroxides, alcohol. The results of solubility analysis are given in Table No.6. It was slightly soluble in water, very slightly soluble in methanol, acetone, soluble in 0.1N HCl, dil.alkali hydroxides, insoluble in alcohol.

ANALYTICAL METHOD FOR THE DETERMINATION OF MESALAMINE

Determination of λmax

The 100µg/ml sample was prepared and scanned between 200 to 400nm. The drug showed maximum absorption at 230nm. So, the λ max of Mesalamine was found to be 230nm.

Preparation of Calibration Curve of Mesalamine in 0.1N HCl.

Table 7: Standard calibration curve data of Mesalamine in pH

CONCENTRATION (µg/ml)		ABSORBANCE
0		0
2		0.228
4		0.459
6		0.594
8		0.785
10		0.995

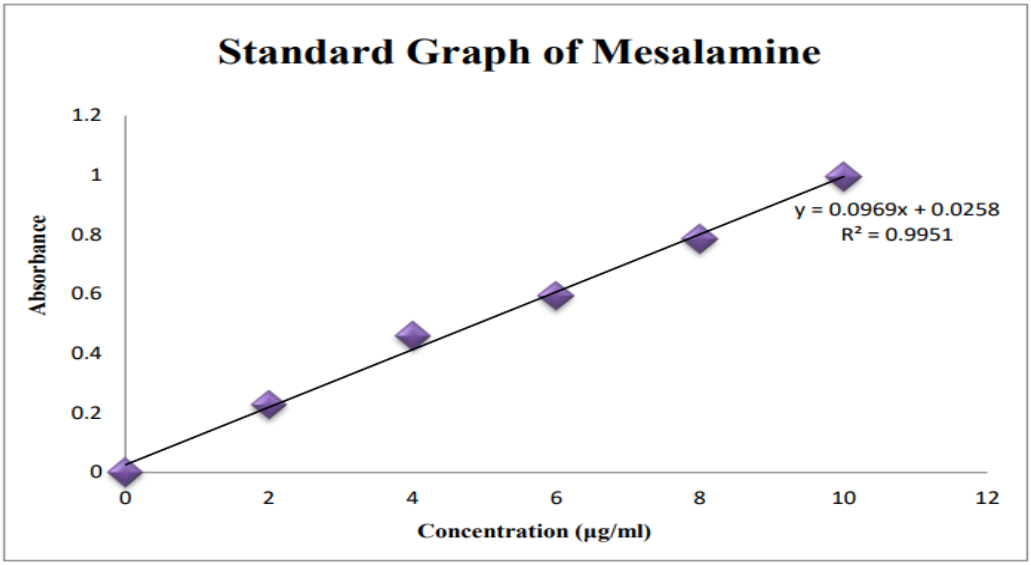

Figure 3:Standard curve of Mesalamine in pH 6.8 Buffer

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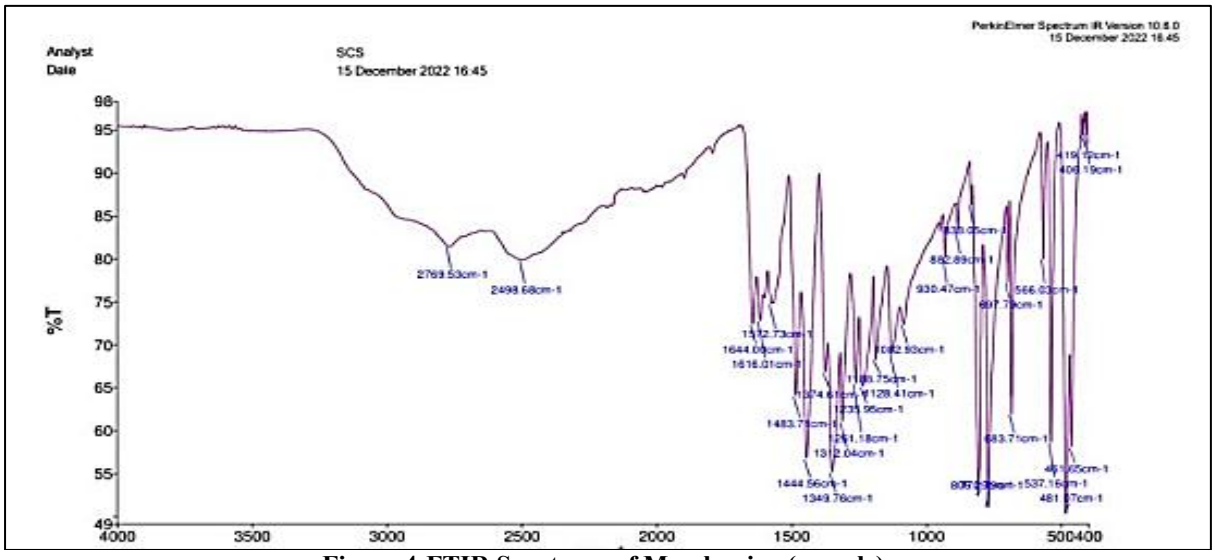

Figure 4:FTIR Spectrum of Mesalamine (sample)

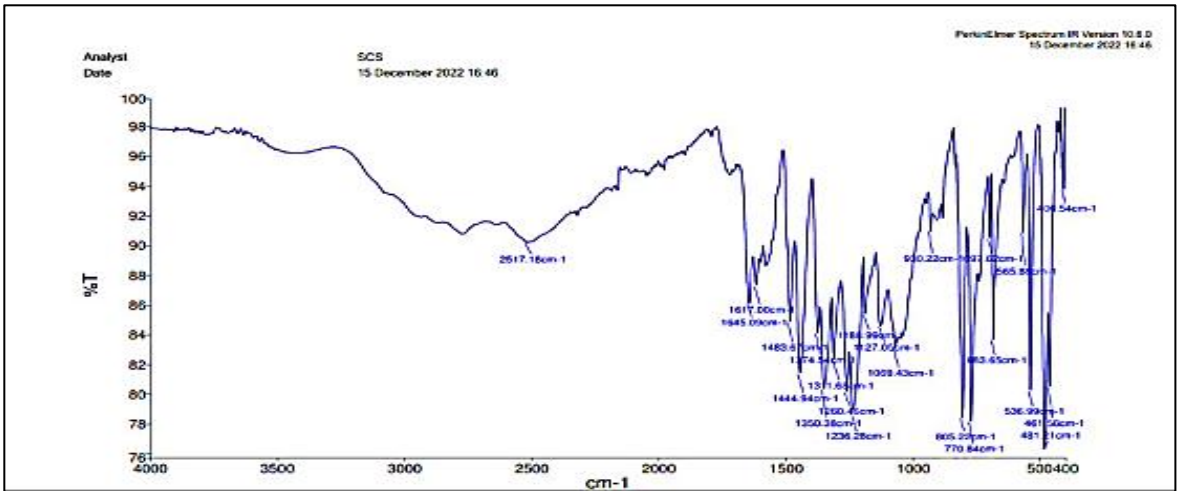

Figure 5: FTIR Spectrum of Mesalamine + Cellulose Acetate Phthalate (CAP)

FTIR

FTIR studies were carried out for the drug [Mesalamine] and for the drug-excipients physical mixtures as shown in Fig. No 4,5 respectively. The results are summarized in Table No. 8. The FTIR spectrum of Mesalamine exhibited peak signals at 1444 cm-1 due to a C=H, stretching 2769 cm-1 due to OH stretching, and 1235 cm-1 due to C-O stretching. There were no significant changes in the frequency of the functional groups of Mesalamine. So, the drug was compatible with all excipients.

Table 8: Comparison of FTIR Spectra of Mesalamine and excipients

Sl. No.	Drug	Functional Groups (cm^{-1})				
		C-O	C-H	C=C	C=O	O-H
1.	Mesalamine	1235	1444	1572	1645	2517
2.	Mesalamine + Cellulose Acetate Phthalate	1236	1444	1483	1644	2769

DSC

The DSC studies were carried out for drug (Mesalamine) and Drug-Polymer physical mixtures. The results are given in Fig. No.6,7. The recorded DSC thermograms showed the profile of Mesalamine with melting point at 284.63˚C. Drug when combined with excipients, showed melting point at 280.32ºC. The melting point remains almost the same, indicated that the drug and excipients are compatible with each other.

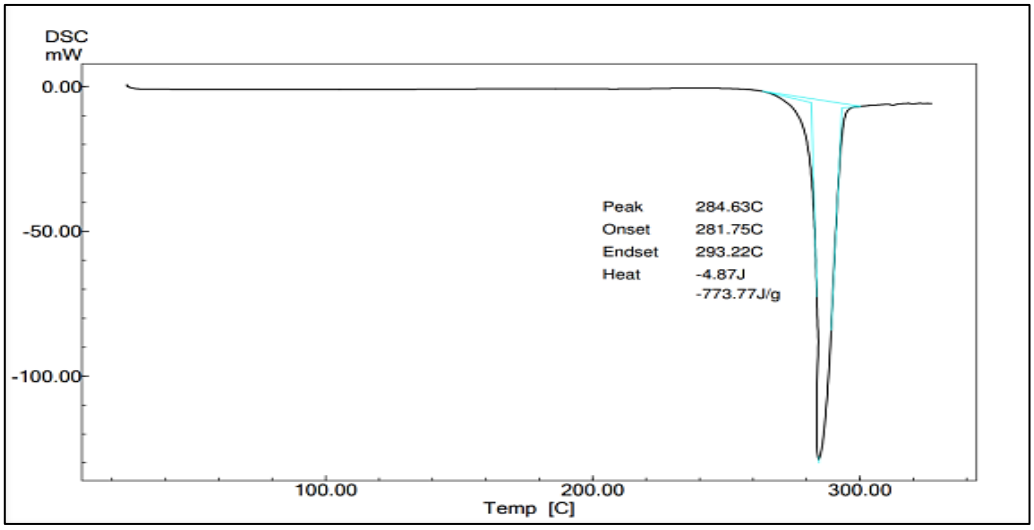

Figure 6: DSC Curve of pure Mesalamine

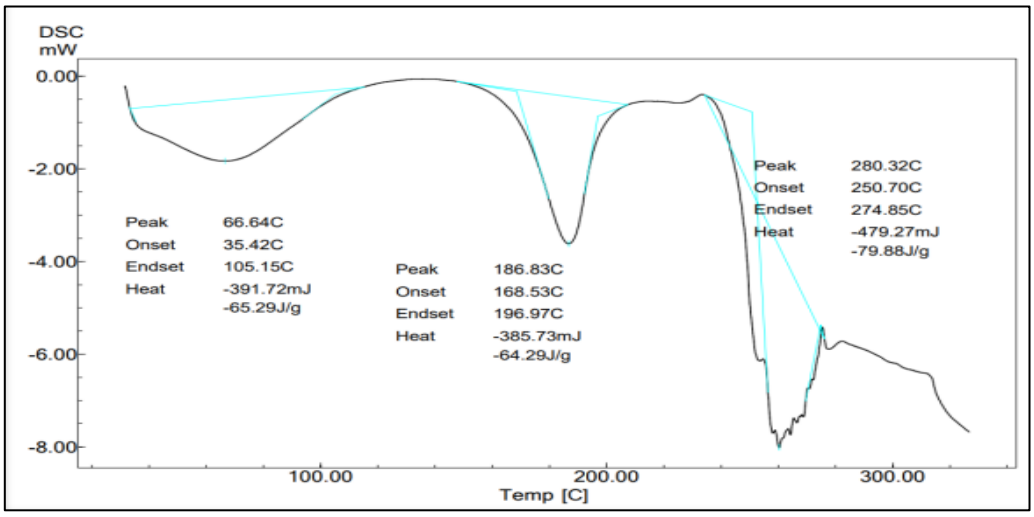

Figure 7: DSC Curve of pure Mesalamine + Cellulose Acetate Phthalate (CAP)

Preparation of Mesalamine Microspheres

EVALUATION

Table 9: Flow Properties of Microspheres

Formulation	Angle of repose	Bulk density	Tapped density	Carr's index	Hausner's ratio
F1	23.54±0.571	0.585±0.004	0.675±0.055	13.33±0.189	1.15±0.040
F2	22.56±1.892	0.655±0.006	0.721±0.011	10.07±0.607	1.10±0.036
F3	24.31±2.257	0.527±0.002	0.603±0.046	12.60±0.346	1.14±0.017
F4	21.65±1.571	0.499±0.003	0.591±0.020	15.56±0.899	1.18±0.029
F5	24.76±0.844	0.436±0.007	0.526±0.027	17.11±0.655	1.21±0.040
F6	26.11±1.076	0.532±0.005	0.610±0.025	12.78±0.321	1.14±0.011
F7	26.24±1.045	0.492±0.002	0.519±0.016	12.32±0.357	1.05±0.028
F8	27.91±1.156	0.534±0.005	0.649±0.032	17.71±0.653	1.21±0.015
F9	25.69±0.991	0.658±0.006	0.729±0.022	10.79±0.410	1.11±0.003
F10	22.90±1.213	0.609±0.007	0.718±0.061	17.89±0.654	1.17±0.015
F11	25.08±2.111	0.546±0.007	0.618±0.009	13.18±0.748	1.13±0.043
F12	23.33±1.318	0.561±0.012	0.659±0.049	14.87±1.121	1.17±0.048
F13	25.76±0.856	0.532±0.005	0.663±0.009	19.75±0.590	1.24±0.027
F14	26.21±0.782	0.576±0.005	0.649±0.032	11.24±0.382	1.12±0.043
F15	26.21±0.143	0.587±0.007	0.675±0.019	13.03±0.178	1.14±0.047
F12	24.36±0.791	0.548±0.007	0.652±0.035	15.95±0.892	1.18±0.016
F17	24.41±0.811	0.598±0.006	0.699±0.035	14.44±1.023	1.16±0.009

Figure 8: Prepared microspheres

Micromeritic Properties

The results of micrometric properties such as bulk density, tapped density, % Compressibility index, Hausner's ratio and angle of repose for the formulations F1 to F17 are shown in the above Table No.9. The value of bulk density ranges from 0.436 to 0.658 for all the formulations and tapped density ranges from 0.603 to 0.729. it was found that the values are less than 1. The % Compressibility index was in the range of 11-1.8. Hausner's ratio was found in 1.5 to 1.24. The values of angle of repose for formulations were found to be in the range of 25-30. Table No.9 suggests that all the values were within the range which indicated a good flow property of formulated microspheres.

Particle size determination

The study investigated how three factors (polymer concentration, stirring speed, and surfactant concentration) affect the particle size of microspheres. The results showed that all three factors influence particle size, with sizes ranging from 98.16μm to 235.23μm. The largest particles were obtained with high polymer concentration, high stirring speed, and low surfactant concentration, while the smallest particles were obtained with low polymer concentration, high stirring speed and low surfactant concentration.

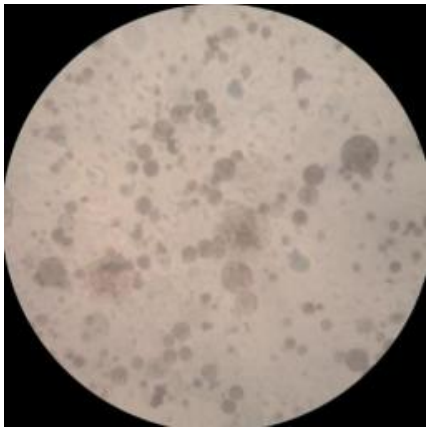

Figure 9: Microscopic view of microspheres

Percentage yield

Percentage yield of all formulations varies from F1 to F17 which are shown in Table No.25 and indicates that F7 shows highest percentage yield of 90.39±0.95%.

Table 10: Percentage yield of microspheres

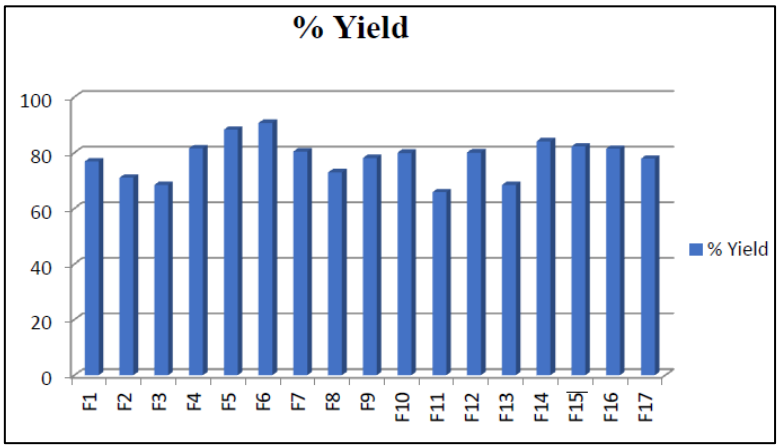

Figure 10: Percentage yield of microspheres

Scanning Electron Microscopy (SEM)

Formulation code	Percentage Yield (%)
F1	76.87±1.00
F2	70.98±2.18
F3	68.44±0.79
F4	81.59±1.18
F5	88.33±1.24
F6	87.71±2.65
F7	90.39±0.95
F8	72.97±1.43
F9	78.15±1.30
F10	79.99±1.11
F11	65.75±1.31
F12	80.11±1.78
F13	68.44±1.71
F14	84.11±0.46
F15	82.25±2.76
F16	81.43±1.51
F17	77.91±0.96

Morphological analysis of the microspheres was carried out using Scanning Electron Microscopy and the result is shown in Fig. No.11

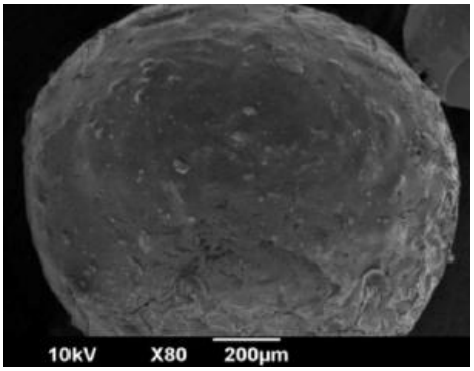

Figure 11: SEM photographs of mesalamine microspheres

***In vitro* drug release studies**

As the polymer concentration increases up to an optimum level (800mg), drug release from microspheres significantly increased, further increase in polymer concentration decreases the drug release, because increasing polymer concentration may result in larger particle size and reduced surface area. Drug release was more rapid and significant from microspheres generated at higher stirring speed spanning from 1500-2500 rpm due to smaller size, larger surface area. Surfactant concentration increases up to an optimum limit (1%), % drug release was found to be decreased. Whereas the concentration of surfactant increases beyond optimum limit, % drug release was found to be decreased. The highest drug release was obtained at optimum level of polymer concentration (800mg), surfactant concentration (1.0%), and stirring speed (2000 rpm).

The results show that formulation of F7 shows maximum release of drug, i.e., 93.59%, compared to other formulations.

Table 11: % CDR of mesalamine microspheres

Cumulative % Drug release													
Media	**pH 1.2**			**pH 6.8**									
TIME	0	1	2	3	4	5	6	7	8	9	10	11	12
F1	0	4.1	6.84	11.34	24.31	33.27	45.81	57.81	63.48	71.77	77.92	84.29	86.08
F2	0	7.29	10.13	12.01	26.2	30.44	39.81	44.21	51.29	63.48	74.38	80.16	88.12
F3	0	6.08	8.16	14.14	21.01	39.96	48.8	54.78	57.31	60.22	69.16	73.21	83.5
F4	0	0.52	0.94	1.17	9.27	20.11	38.43	51.27	63.56	70.93	78.91	85.96	90.7
F5	0	3.21	4.68	5.13	12.4	29.31	36.72	50.37	59.01	62.29	70.16	81.26	85.53
F6	0	2.26	5.1	7.66	15.18	21.49	36.41	48.86	52.53	67.23	73.77	78.19	84.87
F7	0	0.15	0.74	1.06	10.81	22.27	40.19	58.1	66.36	72.18	74.1	87.26	93.59
F8	0	4.71	6.29	9.01	16.01	34.24	45.77	51.42	62.79	69.48	77.99	80.55	85.25
F9	0	4.00	5.03	5.98	18.64	35.5	45.31	51.09	40.72	62.86	67.28	76.75	83.83
F10	0	3.99	5.61	9.94	17.83	20.21	31.76	40.72	59.9	64.47	74.11	83.39	86.56
F11	0	6.33	8.46	10.7	22.1	38.3	49.62	53.16	58.71	61.44	69.78	78.36	81.12
F12	0	0.7	1.07	1.21	6.53	13.45	28.88	41.26	54.32	70	82.33	89.12	91.18
F13	0	4.46	5.55	9.37	11.18	25.19	38.77	47.59	56.82	66.43	72.69	76.9	81.44
F14	0	1.8	2.31	3.04	8.87	26.33	30.84	48.73	60.13	69.95	72.04	76.08	87.06
F15	0	0.82	1.18	1.25	9.44	12.33	27.46	31.98	42.04	59.11	74.58	83.22	92.49
F16	0	0.99	1.01	1.28	6.9	16.87	28.75	36.83	52.17	60.77	74.48	81.07	90.85
F17	0	3.12	3.76	6,42	14.11	29.32	36.29	40.13	53.83	65.94	70.52	74.27	80.19

KINETIC STUDY

To determine the release mechanism that gives the best description to the pattern of drug release, the *in vitro* release data were fitted to zero-order, first order, Hixson Crowell equation and Higuchi matrix model. The release data were also kinetically analysed using the Korsmeyer–Peppas model. The accuracy and prediction ability of the models were compared by calculation of R2 as given in Table No 12. The model giving R2 close to unity was taken as the best fit model. The release exponent values thus obtained were ranged from 1.10 to 2.88. The mechanism of drug released was calculated by applying the kinetic models and it was concluded that the formulations F7 follows the zero-order model and it undergoes super case II Transport mechanism.

Table 12: Kinetic profile of mesalamine microspheres

Formulation Code	Zero order	First order	Higuchi	Hixon crowell	Korsemeyer Peppas	
	R^2	R^2	R^2	R^2	R^2	N
F1	0.9811	0.9519	0.883	0.9668	0.9758	1.36
F2	0.9765	0.9844	0.8685	0.946	0.962	1.20
F3	0.9743	0.9462	0.899	0.9711	0.9616	1.26
F4	0.9524	0.8991	0.8001	0.9324	0.9265	2.47
F5	0.9702	0.9138	0.8136	0.9464	0.9328	1.32
F6	0.9715	0.9186	0.8331	0.946	0.984	2.35
F7	0.9504	0.8917	0.9325	0.9321	0.9464	1.10
F8	0.9731	0.9764	0.9731	0.966	0.9476	1.36
F9	0.9698	0.9729	0.9698	0.9699	0.9227	1.45
F10	0.9655	0.9857	0.9655	0.9231	0.9694	1.39
F11	0.9746	0.9792	0.9746	0.9753	0.9458	2.18
F12	0.9327	0.9407	0.9327	0.8846	0.9281	2.37
F13	0.9686	0.9775	0.8398	0.9538	0.9404	1.37
F14	0.9544	0.9628	0.8086	0.9335	0.9238	2.88
F15	0.9247	0.7745	0.7359	0.843	0.92262	2.23
F16	0.9442	0.8262	0.7652	0.8844	0.9095	2.24
F17	0.9324	0.9735	0.8407	0.9538	0.9492	1.53

Optimization and evaluation of optimized formulation

To obtain the desired response, numerical optimization using the desirability approach was employed to locate the optimal settings of the formulation variables. By setting constraints on the dependent and independent variables the optimized formulation (F7) was developed. The optimized formulation was achieved at (polymer concentration: 800 mg, stirring speed: 2000 rpm, surfactant concentration: 1.0%) suggested by the software with the corresponding desirability (D) value of 0.928. Finally, three batches of optimized formulations were prepared to confirm the validity of the optimal parameters and predicted responses calculated. All the responses were evaluated for each optimized formulation. It can be seen that the experimental values were remarkably close to the design predicted values, which represents factual consistency, reliability, and validity of BBD in colon-targeted delivery of mesalamine microspheres.

Table 13: The optimized formulation (F7) levels, predicted, and observed values.

Independent variables		**Optimized levels**
Polymer concentration (mg) (X_1)		800
Stirring speed (rpm) (X_2)		2000
Surfactant concentration (%) (X_3)		1.00
Dependent variables	**Predicated responses**	**Observed responses**
Particle size (µm) (Y_1)	156.96	152.65
Entrapment efficiency (%)	84.48	87.55
Drug release in 3 hrs (%)	1.18	1.06
Drug release in 12 hrs (%)	91.49	93.59

Table 14: Stability Study of optimized formulation.

Duration	**Entrapment Efficiency (%)**	**% Cumulative drug release**	
		pH 1.2 (3 hrs)	pH 6.8 (12 hrs)
Initial	87.55	1.06	93.59
3rd Month	85.91	2.65	90.87

STABILITY STUDIES

The selected formulation F7 was subjected to stability study. Initial and third month studies were done and results were mentioned in Table No.14. There were no significant changes in the entrapment efficiency and drug release characteristics which provide evidence for better stability of the prepared formulations in accelerated stability conditions. The stability studies will be continued further up to six months.

IV. CONCLUSION

Mesalamine is quickly absorbed in the upper digestive tract, but coating it with cellulose acetate phthalate prevents this. Microencapsulation with a polymer using solvent evaporation allows the drug to be released specifically in the colon, reducing side effects. Conventional mesalamine therapies require frequent dosing, leading to low patient compliance and potential side effects. Microencapsulation of the drug enables less frequent dosing (once a day), improving patient compliance.

From this study, it is concluded that that the microspheres ensures a better alternative to the conventional dosage forms and proves to be promising for the colon targeted delivery for the treatment of Ulcerative colitis.

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