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DEVELOPMENT AND EVALUATION OF COLON TARGETED DRUG DELIVERY FOR ULCERATIVE COLITIS

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ABSTRACT

The main objective of this study was to formulate mesalamine loaded alginate microspheres for local treatment of ulcerative colitis. The microspheres were prepared by ionic gelation method. Box Behnken design using design expert software was employed in formulating and optimizing the microspheres. Microspheres were evaluated for particle size, shape and entrapment efficiency. The drug release of microspheres showed a longer residence time in the colon due to better mucoadhesion properties of sodium ALG. Therefore mesalamine-loaded alginate microspheres can be potential delivery system for local treatment of ulcerative colitis.

Keywords: Mesalamine, Ulcerative colitis, Sodium alginate, Box Behnken design

INTRODUCTION

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 [1]. 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 [2].

Mesalamine is widely used in long-term treatment of ulcerative colitis by its topical mode of action on the inflammation in colonic mucosa. However, it has been reported that 5-ASA is extensively absorbed and metabolized in the upper gastrointestinal tract by first pass metabolism and is not made available to the desired site colon. This results not only in systemic side effects but also in lowering the dose reaching the colon with the subsequent decreased probability of therapeutic success [3]. Its mean half-life is 2–15 h. Single unit dosage forms for colonic delivery may suffer from the disadvantage of unwarranted disintegration of the formulation due to high inter- and intra-subject viability and poor reproducibility, which may lead to loss of local therapeutic action in the colon. Therefore, little emphasis is being laid on the preparation of single unit dosage forms in comparison to multi-particulate delivery system due to their possible benefits, like better bioavailability, decreased risk of local irritation and predictable gastric emptying [4,5]. The aim of present study was made to formulate mesalamine-loaded alginate microspheres .Mucoadhesive microspheres for the treatment of colon diseases is recently wide in use. Bioadhesive delivery could benefit the controlled release of drugs. Here alginate-based microspheres for the colon-specific delivery of Mesalamine have been developed. Sodium alginate (ALG) being a nontoxic, biocompatible, and biodegradable polymer comes under the group of natural polysaccharides present in the seaweed. [6,7]

MATERIALS AND METHODS

Materials

Mesalamine, sodium alginate,calcium chloride were procured from Carbanio.com. Isopropyl alcohol was purchased from We Associates, Kerala. All other reagents and solvents were of the highest analytical grade commercially available.

Methodology

EXPERIMENTAL DESIGN

Optimization of colon-specific microspheres

Box Behnken Design (BBD) was used to optimize mesalamine microspheres for colon-targeted drug delivery employing Design Expert Software. The concentration of Polymer (X1), Stirring speed (X2), concentration of cross-linking agent (X3) were chosen as independent variables at low, medium and high levels respectively, shown in Table No.1. Particle size (Y1), drug entrapment efficiency (Y2), percentage yield (Y4) were chosen as response factors [8].

Table no 1: Box Behnken Design layout for optimization of mesalamine microsphere

Formulation Code	Run order	X_1-Polymer conc. (%)	X_2–Stirring speed (rpm)	X_3–Cross linking agent. (%)
F1	1	6	1000	5
F2	2	4	1500	5
F3	3	2	1500	4
F4	4	4	1000	4
F5	5	6	1000	3
F6	6	6	500	4
F7	7	4	1000	4
F8	8	4	1500	3
F9	9	4	500	5
F10	10	6	1500	4
F11	11	2	1000	3
F12	12	4	1000	4
F13	13	2	1000	5
F14	14	4	1000	3
F15	15	4	1000	4
F16	16	4	1000	4
F17	17	2	500	4

FORMULATION OF MICROSPHERES

Microspheres were prepared by ionic gelation method. Initially sodium alginate was dissolved in water to obtain different concentration and drug was dissolved in 0.1N HCl. This solution was added to the solution of sodium alginate with constant stirring. Calcium chloride solution was prepared separately. From a constant height, calcium chloride solution was added dropwise into sodium alginate solution. The system was kept under constant stirring. After 2 hours of stirring, the solvent was decanted and the product was washed several times with distilled water, dried [9].

Table no 2: Formulation composition of mesalamine microspheres

Formulation code	Ingredients		
	Mesalamine (mg)	Sodium alginate(%)	Calcium chloride(%)
F1	400	6	5
F2	400	4	5
F3	400	2	4
F4	400	4	4
F5	400	6	3
F6	400	6	4
F7	400	4	4
F8	400	4	3
F9	400	4	5
F10	400	6	4
F11	400	2	3
F12	400	4	4
F13	400	2	5
F14	400	4	3
F15	400	4	4
F16	400	4	4
F17	400	2	4

EVALUATION OF MICROSPHERES

Particle size 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) [10].

Percentage yield of Microspheres

The prepared microspheres of all batches were accurately weighed. The weighed 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 [10].

Percentage yield = (Practical yield/Theoretical yield) × 100

Shape and surface morphology

The shape and surface morphology of Mesalamine microspheres were investigated using scanning electron microscopy [10].

Entrapment efficiency

The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 230 nm against appropriate blank. The amount of drug entrapped in the microspheres was calculated by the following formula [10,11,12].

Percentage drug entrapment = Actual drug content / Theoretical drug content X 100

***In vitro* drug release**

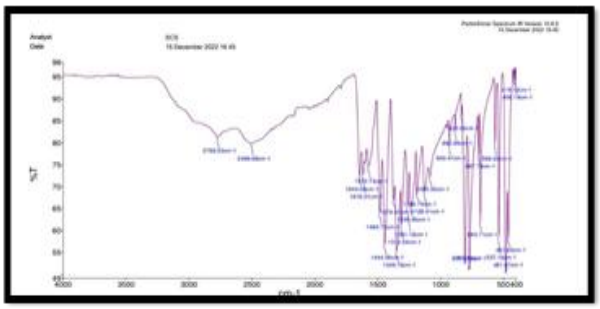
Dissolution was performed using United States Pharmacopoeia (USP) type I (basket) apparatus. The drug loaded microspheres equivalent to 100 mg of mesalamine were introduced into 900 ml of dissolution medium which was maintained at 37 ± 0.5^0 C and stirred at 100rpm. The dissolution was carried out in 0.1N HCl for the first 3 hrs followed by pH 6.8 buffer for the next remaining 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 throughout the study by replacing an equal volume of fresh dissolution medium. 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. **[13].**

STABILITY STUDIES

The stability studies were carried out as per ICH guidelines. The optimized formulation was subjected to accelerated stability studies for a period of 3 months at a temperature of 40ºC±2℃ and Relative Humidity (RH) 75%RH±5% RH in a stability chamber. Samples were withdrawn at an interval of time and analyzed suitably for Entrapment efficiency and dissolution characteristics.

RESULTS AND DISCUSSION

- **FTIR spectra**



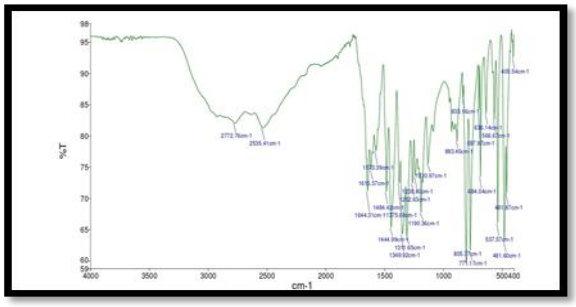
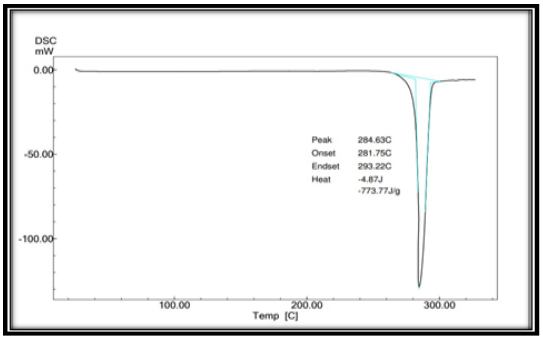


Fig No.1: FTIR spectrum of Mesalamine(sample) **Fig no.2: FTIR spectrum of Mesalamine + Excipients**

DSC



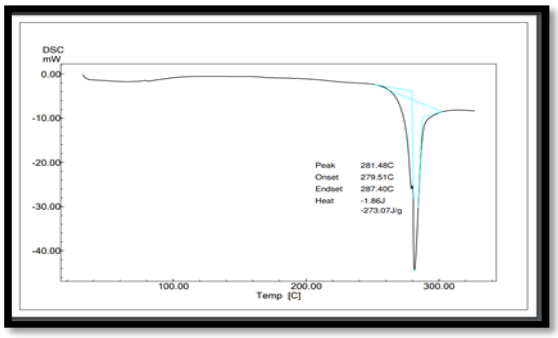


Fig.No.3: DSC Curve of pure Mesalamine **Fig. No.4: DSC Curve of pure Mesalamine + excipients**

Preparation of Mesalamine Microspheres

Optimization using Box Behnken design

In the Box-Behnken design, a total of 17 formulations were proposed by Design expert software for three factors such as the polymer concentration (X1), stirring speed (X2) and concentration of cross-linking agent (X3), which were varied at three different levels (−1, 0 and 1). The effects of independent variables (factors) on the particle size (µm), drug entrapment efficiency (%), percentage yield(Y3) were examined as optimization response parameters in this study. The observed values of independent variables are given in Table No.3.

Table no 3: Observed values of responses

Formulation code	Particle size (µm)	Entrapment efficiency (%)	Percentage Yield (%)
F1	145.79	84.12	76.87±1.00
F2	121.36	86.5	70.98±2.18
F3	106.89	79.16	68.44±0.79
F4	132.29	81.32	81.59±1.18
F5	182.79	83.41	88.33±1.24
F6	135.23	85.95	87.71±2.65
F7	112.65	87.55	90.39±0.95
F8	143.96	74.45	72.97±1.43
F9	148.21	81.9	78.15±1.30
F10	130.53	83.51	79.99±1.11
F11	100.34	72.62	65.75±1.31
F12	158.12	82.5	80.11±1.78
F13	107.18	85.33	68.44±1.71
F14	157.55	74.75	84.11±0.46
F15	160.12.	79.58	82.25±2.76
F16	156.36	83.78	81.43±1.51
F17	98.16	77.48	77.91±0.96

Table no :4 Micromeritic Properties

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
F16	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

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.4. 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 10-19. Hausner's ratio was found in 1.05to 1.24. The values of angle of repose for formulations were found to be in the range of 21-27. Table No.4 suggests that all the values were within the range which indicated a good flow propertyof formulated microspheres.

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 was developed. The optimized formulation was achieved at (polymer concentration: 6.0%, stirring speed: 1500 rpm, cross linking agent: 5.0%) suggested by the software with the corresponding desirability (D) value of 0.894. 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

Scanning Electron Microscopy (SEM)

Morphological analysis of the microspheres was carried out for the optimized batch of microspheres using Scanning ElectronMicroscopy and the result is shown in Fig. No.5. The SEM photograph reveals that the microspheres were spherical in shape.

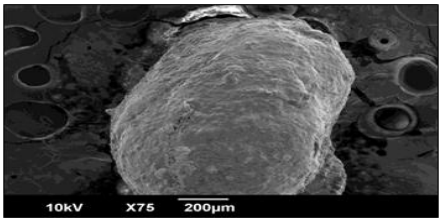


Fig.No.5: SEM image of microspheres

***In vitro* drug release studies**

In-vitro dissolution studies were performed for the optimized formulation. As per the results (Table no 8) of dissolution study formulations showed 92.08% release at 12 th hour. This showed that the drug release was sustained for 8 to 12 hr.

Table No 8: % 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
F	0	4.1	6.84	11.34	24.31	33.27	45.81	57.81	63.48	71.77	80.92	88.29	92.08

Stability study

The results of stability studies indicate no significant changes in the drug release characteristics which provide evidence for better stability of the prepared formulations in accelerated stability conditions.

Table No 9: Stability studies

Duration	**% cumulative drug release** **pH 6.8(12 hours)**
Initial	86.08
3 months	83.56

CONCLUSION

The purpose of this work was to design mesalamine loaded colon-specific delivery system. To achieve site-specific drug delivery to the colon, mesalamine microspheres were formulated. Mesalamine microspheres were prepared by by ionic gelation. The mucoadhesive nature of sodium alginate controls the drug release up to 12 hour. Therefore, mesalamine loaded alginate microsphere can be a potential drug delivery system for treatment of colon disease.

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