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FORMULATION AND EVALUATION OF COLON TARGETING MICROSPHERES CONTAINING SULFASALAZINE FOR IBD

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Abstract:

The main objective of the work was to prepare and evaluate sulfasalazine loaded microspheres for sustained delivery for the treatment of inflammatory bowel disease. Sulfasalazine has crystalluria, thrombocytopenia, and megaloblastic anemia as side effects, so to reduce side effect microspheres were prepared. The sulfasalazine microspheres were prepared by inotropic gelation method by optimizing process parameters such as concentration of calcium chloride, agitation speed, and time of agitation. The concentration of polymer sodium alginate was varied. Among the all formulations, the best formulation was considered by comparing process parameters such as the entrapment efficiency, drug content, in vitro drug release studies, scanning electron microscope analysis, and zeta potential. The prepared microspheres were stable, spherical particles and showed favorable release profiles in simulated colonic fluid. However, additional evaluation of these carriers can be performed for their probableto treat colonic diseases, as a future scope.

Key words:*Sulfasalazine, Microspheres, Inflammatory bowel disease, Development, Characterization*

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INTRODUCTION:

Inflammatory bowel disease (IBD) is the cover name for at least two diverse entities, namely ulcerative colitis (UC) and Crohn's disease (CD) each having its own spectrum of presentation and medical course. IBD currently consists of UC and CD. The two diseases are essentially different in that CD is generally a transmural inflammation, involving the whole thickness of the bowel wall, while UC is usually confined to the mucosa. In addition, CD can involve anywhere from the mouth to the anal canal, while UC affects the colon almost exclusively. There are also genetic predispositions which differ among the two conditions [1].

Crohn's Disease, a standout amongst the most incessant types of incendiary sickness around the world, is portrayed by the development of strictures, fistulas, ulcers, and granulomas in the mucosa. Despite the fact that the CD's gastrointestinal indication can basically influence the terminal ileum district, it can likewise bargain any area from the mouth to the rectum of influenced quiet. The clinical indications of CD can incorporate looseness of the bowels or wicked the runs, lack of healthy sustenance, stomach torment, and weight reduction. Additional intestinal discoveries, for example, arthropathy or skin issue, once in a while happen. In any case, appearances on skin, muscle, or bone of metastatic Crohn's sickness can really prompt acknowledgment of mysterious intestinal malady. All in all, CD has a hereditary foundation and the main degree relatives of influenced people have a fivefold more serious danger of building up the malady [2-3].

Ulcerative colitis is a new form of IBD characterized by superficial ulcerations, granularity, and a vascular pattern. In contrast with the inflammation found in Crohn's disease transmural and being capable to occur throughout the total gastrointestinal tract inflammation in UC is limited to themucosal layer of the colon [4-5].

While the correct reason for IBD isn't completely understood, it is known to include collaboration between qualities, the resistant framework, and ecological variables. The resistant framework normally assaults and executes outside trespassers, for example, microscopic organisms, infections, parasites, and different microorganisms. Be that as it may, in individuals with IBD, the insusceptible framework mounts a wrong reaction to the intestinal tract, bringing about irritation.

This strange resistant framework response happens in people who have acquired qualities that make them susceptible to IBD. Unidentified ecological variables fill in as the "trigger" that starts the hurtful invulnerable reaction in the digestion tracts.

Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles" (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Microspheres are sometimes referred to as microparticles. Biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin, the synthetic polymer include poly lactic acid and polyglycolic acid. The solvents used to dissolve the polymeric materials chosen according to the polymer and drug solubility and stabilities, process safety and economic considerations [6]. Microspheres for oral use have been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation.

Microspheres comprise a significant part of this particulate drug delivery system by virtue of their small size and proficient carrier characteristics. However, the achievement of this new drug delivery system is limited due to their short residence time at the site of absorption. It would so be beneficial to have means for providing an intimate contact of the drug delivery system with absorbing gastric mucosal membranes.

Microspheres are typicallyfree powders consisting of proteins or synthetic polymers that are biodegradable in nature and preferably having a particle size less than 200μm. Inflammatory bowel disease (IBD) is a chronic relapsing and remitting inflammatory disorder of the small intestine and colon. Ulcerative colitis (UC) and Crohn's disease (CD) are the two main types of IBD. Inflammatory bowel disease (IBD) is a chronic relapsing and remitting inflammatory disorder of the small intestine and colon. IBD includes ulcerative colitis (UC) and Crohn's disease (CD), and it is a main reason for the expansion of colon cancer, referred to as colitis associated cancer (CAC). Oral colon targeted microsphere-based drug delivery system containing sulfasalazine will be prepared, optimized and characterized.

MATERIAL AND METHODS:

Preparation of chitosan microspheres of Sulfasalazine:

Chitosan microspheres were prepared by ionotropic gelation method [46]. Chitosan stock solution (1% w/v) was prepared by dissolving chitosan in acetic acid (1% v/v) at room temperature. The drug (2-5 mg) was dissolved in chitosan solution. 1% Sodium tripolyphosphate solution was prepared in water. Sodium tripolyphosphate solution was added drop wise with a syringe to chitosan solution while stirring. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Microspheres were obtained which was air dried for twenty-four hours followed by oven drying for six hours at 40˚C.

Table 1: Formulations of chitosan microspheres prepared

Sr. No	Formulation Code	Sulfasalazine (mg)	Chitosan (mg)	STPP (mg)
1.	F1	10	250	500
2.	F2	10	250	750
3.	F3	10	250	1000
4.	F4	10	500	500
5.	F5	10	500	750
6.	F6	10	500	1000

Coating of chitosan microspheres:

Microsphereswere coated with Eudragil S-100 (ES) using solvent evaporation method. Microspheres (50 mg) were dispersed in 10 mL of coating solution prepared by dissolving 500 mg of eudragit S-100 in ethanol: acetone (2:1) to give 5:1 (coat: core ratio). This organic phase was then poured in 70 mL of light liquid paraffin containing 1% wt/vol Span 80. The system was maintained under agitation speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-hexane, and dried in desiccators [47].

Evaluation of microspheres

There are many formulations and process variables involved in mixing step and all these can affect characteristics of blend produced, bulk density, true density and percent compressibility index have been measured which are given in table.

Bulk density

Bulk density is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup.

Procedure: -

A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, Vo, to the nearest graduated unit. Calculate the bulk density, in gm per ml gm/ml, by the formula

Bulk density = Bulk Mass/ Bulk Volume

Compressibility index (Carr's index):

Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities. Carr's index a material having values of less than 20% to 30% is defined as the free flowing material.

It can be calculated as per given formula:

$$\% \text{ CI} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} x100$$

Hausner ratio:
It indicates the flow properties of the powder and it can be measured by the ratio of tapped density to bulk density.

Hausner ratio = Tapped density / Bulk Density

Percentage Yield:
The prepared microspheres F1-F6 were collected and weighed from each formulation. The percentage yield (%) was calculated using formula given below:

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

Entrapment Efficiency:
Amount of Sulfasalazine in each formulation was calculated according to procedure given below [48]:10 mg of chitosan microspheres from each batch were accurately weighed. The powder of chitosan microspheres were dissolved in 10 ml 7.4 pH Phosphate Buffer and centrifuge at 1000 rpm. This supernatant solution is than filtered through Whatman filter paper No. 44. After filtration, from this solution 0.1 ml was taken out and diluted up to 10 ml with 7.4 pH Phosphate Buffer. The supernant was analyzed for drug content by measuring the absorbance at 350.50nm.

Measurement of mean particle size:
The mean particle size of the nanoparticle was determined by Photo Correlation Spectroscopy (PCS) on a submicron particle size analyzer **(**Malvern particle size analyser**)** at a scattering angle of 90°. A sample (0.5mg) of the nanoparticle suspended in 5 ml of distilled water was used for the measurement.

Determination of zeta potential:
The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern particle size analyzer) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate.

***In-vitro* Release Studies:**
***In vitro* drug release in gastrointestinal fluids of different pH**
The prepared microspheres were evaluated for *in vitro* drug release. The drug release studies were carried out using USP I Basket type dissolution test apparatus. The dissolution study was carried out in 900 ml dissolution medium which was stirred at 100 rpm maintained at 37±0.2°C. The scheme of using the simulated fluids at different timing was as follows:

- *1st hour:* Simulated gastric fluid (SGF) of pH 1.2.
- *2nd and 3rd hour:* Mixture of simulated gastric and Intestinal fluid of pH 4.5.
- *4th to 5th hour:* Simulated intestinal fluid (SIF) of pH 6.8.
- *6th hour and onward:*SIF pH 7.4

A weighed quantity of formulation (equivalent to 30mg) was filled in capsule and kept in basket of dissolution apparatus with dissolution media (900 ml) at 37±0.2°C. Samples were withdrawn at different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 5ml by media. The samples withdrawn were assayed spectrophotometrically at 242.0 nm for percent of release Sulfasalazine using UV visible spectrophotometer. The release of Sulfasalazine was calculated with the help of Standard curve of Sulfasalazine.

RESULTS AND DISCUSSION:

The loose bulk density (LBD) and tapped bulk density (TBD) of the microspheres of different formulations were evaluated before the compression of powders in to tablets. The bulk density and the tapped density for all the formulations varied from 0.331 to 0.348gm/cm^3 and 0.425 to 0.457gm/cm^3 respectively. The values obtained lies within the acceptable range. The difference exists between the bulk density and tapped density found to be very few. This result helps in calculating the % compressibility of the powder. The result of Hausner's ratio of all formulations ranges from 1.256 to 1.313 which indicates that the flow ability of all the formulation.

The results of the Compressibility index of all the formulations ranges from 20.41% to 25.39%. Results clearly showed that the flow ability of all the formulations was good and also the powder had good compressibility. Percentage yield of different formulation was determined by weighing the microspheres after drying. The percentage yield of different formulation was in range of 69.85±0.26 – 79.85±0.25%. The drug entrapment of different formulations was in range of 68.78±0.24 to 75.65±0.15% w/w. This is due to the Mucoadhesion

characteristics of chitosan that could facilitate the diffusion of part of entrapped drug to surrounding medium during preparation of Sulfasalazine microspheres. The maximum percentage yield and entrapment efficiency was found formulation F4. The optimized formulation among other batches subjected to further studies.The results of measurement of mean particle size of optimized formulation F4 microspheres were found 185.65 nm respectively. Results of zeta potential of optimized formulation F4 microspheres was found to be -30.50 mV respectively**.**

Table 2: Result of flow properties of prepared sulfasalazine microspheres

F. Code	Bulk density(gm/cm^3)	Tapped density(gm/cm^3)	Compressibility index	Hausner ratio
F1	0.345	0.452	23.67	1.310
F2	0.348	0.457	23.85	1.313
F3	0.335	0.449	25.39	1.340
F4	0.347	0.436	20.41	1.256
F5	0.325	0.425	23.53	1.308
F6	0.331	0.436	24.08	1.317

Table 3: Percentage Yield for Different Formulation

Formulation	Percentage Yield	% Entrapment Efficiency
F1	76.65±0.35	68.89±0.35
F2	75.61±0.45	71.12±0.25
F3	69.85±0.26	72.65±0.20
F4	79.85±0.25	75.65±0.15
F5	68.78±0.15	69.98±0.36
F6	69.95±0.36	68.78±0.24

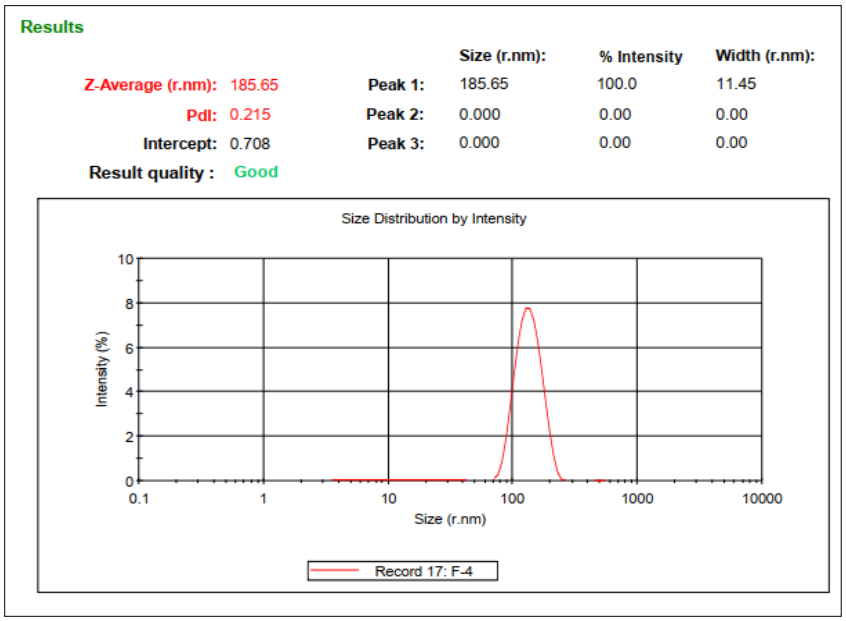

Figure 1: Particle size data of chitosan microspheres (F4)

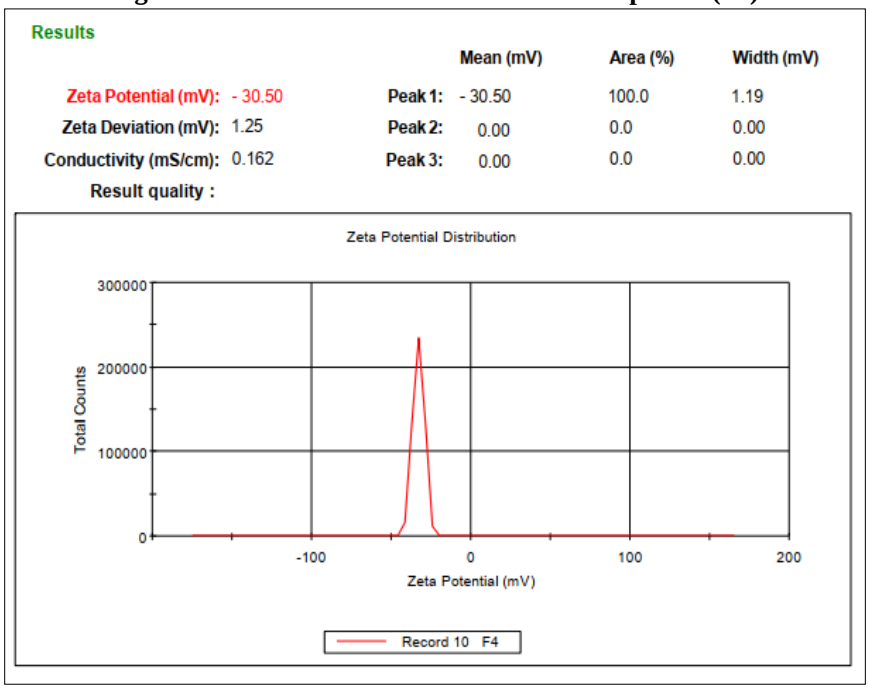

Figure 2: Zeta potential data of chitosan microspheres (F4)

Table 4: Cumulative % drug release of sulfasalazine from plain and Eudragit S100 coated microspheres at different pH

S. No.	Dissolution medium	Time (hrs)	% Cumulative Drug Release	
			Chitosan Microspheres	Eudragit S100 Coated Microspheres
1	SGF (pH 1.2)	1	13.36	3.45
2		2	26.65	4.65
3	SGF+SIF(pH 4.5)	3	38.78	7.32
4		4	42.25	8.45
5		5	55.65	13.32
6	SIF (pH 6.8)	6	63.32	18.85
7		7	69.98	20.14
8	SIF (pH 7.4)	8	73.32	42.23
9		9	79.98	56.65
10		10	82.23	69.98
11		12	85.45	88.78

Table 5: Regression Analysis Data of microspheres Formulation

Formulation	Zero order	First order	Higuchi plot	Pappas plot
F4	y = 8.012x – 18.45 R^2 = 0.888	y = -0.073x + 2.224 R^2 = 0.758	y = 34.12x - 50.08 R^2 = 0.781	y = 1.401x +0.287 R^2 = 0.897

CONCLUSION:

Microspheres loaded sulfasalazine have been prepared by easy emulsification method followed by cross-linking method. The variables such as drug: polymer ratio and concentration of glutaraldehyde were optimized on the basis of particle size, entrapment efficiency. The prepared microspheres were stable, spherical particles and showed favorable release profiles in simulated colonic fluid. However, additional evaluation of these carriers can be performed for their probableto treat colonic diseases, as a future scope.

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