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FORMULATION, DEVELOPMENT AND EVALUATION OF COLON TARGETTING MICROSPHERE OF OLSALAZIN

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Abstract

Olsalazine reduces the bowel inflammation, diarrhea (stool frequency), rectal bleeding, and abdominal pain. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release. The aim of present work to formulate and characterize colon targeting microsphere of Olsalazine. The maximum percentage yield and entrapment efficiency was found formulation F3. The optimized formulation among other batches subjected to further studies. The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. Results of zeta potential of optimized formulation F3 microspheres were found to be -35.48 mV respectively**.** 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 probable to treat colonic diseases, as a future scope.

Key words: Olsalazine, Colon targetting microsphere, Formulation, Evaluation

Introduction

Targeted drug delivery into the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, Crohn's disease, amebiosis, colonic cancer, local treatment of colonic pathologies, and systemic delivery of protein and peptide drugs[1-2]. The colon specific drug delivery system (CDDS) should be capable of protecting the drug en route to the colon i.e. drug release and absorption should not occur in the stomach as well as the small intestine, and neither the bioactive agent should be degraded in either of the dissolution sites but only released and absorbed once the system reaches the colon[3]. The colon is believed to be a suitable absorption site for peptides and protein drugs for the following reasons; (i) less diversity, and intensity of digestive enzymes, (ii) comparative proteolytic activity of colon mucosa is much less than that observed in the small intestine, thus CDDS protects peptide drugs from hydrolysis, and enzymatic degradation in duodenum and jejunum, and eventually releases the drug into ileum or colon which leads to greater systemic bioavailability[4]. And finally, because the colon has a long residence time which is up to 5 days and is highly responsive to absorption enhancers[5].

Oral route is the most convenient and preferred route but other routes for CDDS may be used. Rectal administration offers the shortest route for targeting drugs to the colon. However, reaching the proximal part of colon via rectal administration is difficult. Rectal administration can also be uncomfortable for patients and compliance may be less than optimal[6]. Drug preparation for intrarectal administration is supplied as solutions, foam, and suppositories. The intrarectal route is used both as a means of systemic dosing and for the delivery of topically active drug to the large intestine. Corticosteroids such as hydrocortisone and prednisolone are administered via the rectum for the treatment of ulcerative colitis. Although these drugs are absorbed from the large bowel, it is generally believed that their efficacy is due mainly to the topical application. The concentration of drug reaching the colon depends on formulation factors, the extent of retrograde spreading and the retention time. Foam and suppositories have been shown to be retained mainly in the rectum and sigmoid colon while enema solutions have a great spreading capacity[7].

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[8].

Olsalazine, an anti-inflammatory medicine, is used to treat ulcerative colitis (a condition which causes swelling and sores in the lining of the colon [large intestine] and rectum). Olsalazine reduces the bowel inflammation, diarrhea (stool frequency), rectal bleeding, and abdominal pain.

Microspheres have played a vital role in the development of controlled and or sustained release drug delivery systems. Microspheres have been of particular interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled drug release. The aim of present work to formulate and characterize colon targeting microsphere of Olsalazine.

Material and Methods

Preparation of chitosan microspheres of Olsalazine

Chitosan microspheres were prepared by ionotropic gelation method[9].

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	Olsalazine (mg)	Chitosan (mg)	STPP (mg)
1.	F1	100	250	500
2.	F2	100	250	750
3.	F3	100	250	1000
4.	F4	100	500	500
5.	F5	100	500	750
6.	F6	100	500	1000

Coating of chitosan microspheres

Microspheres were 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[10].

Evaluation of microspheres[11-13]

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:

$$C.I. = \frac{\text{Tapped density- Bulk density}}{\text{Tapped density}} x100$$

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 Olsalazine in each formulation was calculated according to procedure given below [50]:

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 whatmann 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 210.0nm.

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 microspheress was measured on a zeta sizer (Malvern particle size analyser) 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 210.0 nm for percent of release Olsalazine using UV visible spectrophotometer. The release of Olsalazine was calculated with the help of Standard curve of Olsalazine.

Results and Discussion

The objective of the present study is on the formulation of Olsalazine loaded Chitosan microsphere coated with eudragit S-100 in the treatment of Inflammatory Bowel Disease. The prepared microspheres were evaluated for Flow properties, Percentage yield, Drug entrapment Zeta potential, Scanning Electronic Microscopy and In vitro drug release study.

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 0.345 to 0.372gm/cm^3 and 0.458 to 0.489gm/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 1.301 to 1.328. Results of Hausner's ratio of all formulations were shown in Table no 7.1 which indicates that the flow ability of all the formulation.

The results of the Compressibility index of all the formulations ranges from 22.69% to 24.67%. Results of Compressibility index of all the formulations were shown in the Table no 7.1. 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 68.89±0.25–75.56±0.14%.

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 Olsalazine microspheres.

The maximum percentage yield and entrapment efficiency was found formulation F3. The optimized formulation among other batches subjected to further studies.

The mean size of the microspheres was determined by photo correlation spectroscopy (PCS) on a submicron particle size analyzer (Particle Size Analyzer from Malvern) at a scattering angle of 90°C. A sample (0.5mg) of the microspheres suspended in 5 ml of distilled water was used for the measurement. The results of measurement of mean particle size of optimized formulation F3 microspheres were found 135.78 nm respectively.

The zeta potential of the drug-loaded microspheres was measured on a zeta sizer (Malvern Instruments) by determining the electrophoretic mobility in a micro electrophoresis flow cell. All the samples were measured in water at 25°C in triplicate. Results of zeta potential of optimized formulation F3 microspheres was found to be -35.48 mV respectively**.**

The average particle size of microspheres was found 130.25, 135.65 and 136.54nm after 1, 2 and 3 month of storage at 4.0 ±0. 2°C while at 25-28±2°C the average vesicle size was found 155.45, 168.85 and 175.85 nm after 1, 2 and 3 month of storage. % EE in microspheres formulation was 74.65, 73.25 and 69.15 after 1, 2 and 3 month of storage at 25-28±2°C while there were no significant changes in % EE and physical appearance in microspheres formulation was observed after 3 month of storage at 4ºC.

Table 2: Result of flow properties of prepared Olsalazine microspheres

F. CODE	BULK DENSITY(GM/CM³)	TAPPED DENSITY(GM/CM³)	COMPRESSIBILITY INDEX	HAUSNER RATIO
F1	0.345	0.458	24.67	1.328
F2	0.352	0.462	23.81	1.313
F3	0.354	0.467	24.20	1.319
F4	0.365	0.475	23.16	1.301
F5	0.372	0.489	23.93	1.315
F6	0.368	0.476	22.69	1.293

Table 3: Percentage Yield for Different Formulation

Formulation	Percentage Yield	% Entrapment Efficiency
F1	68.89±0.25	63.32±0.35
F2	70.23±0.32	70.45±0.25
F3	75.56±0.14	75.85±0.65
F4	65.85±0.22	69.85±0.14
F5	71.12±0.15	67.74±0.24
F6	69.95±0.18	68.98±032

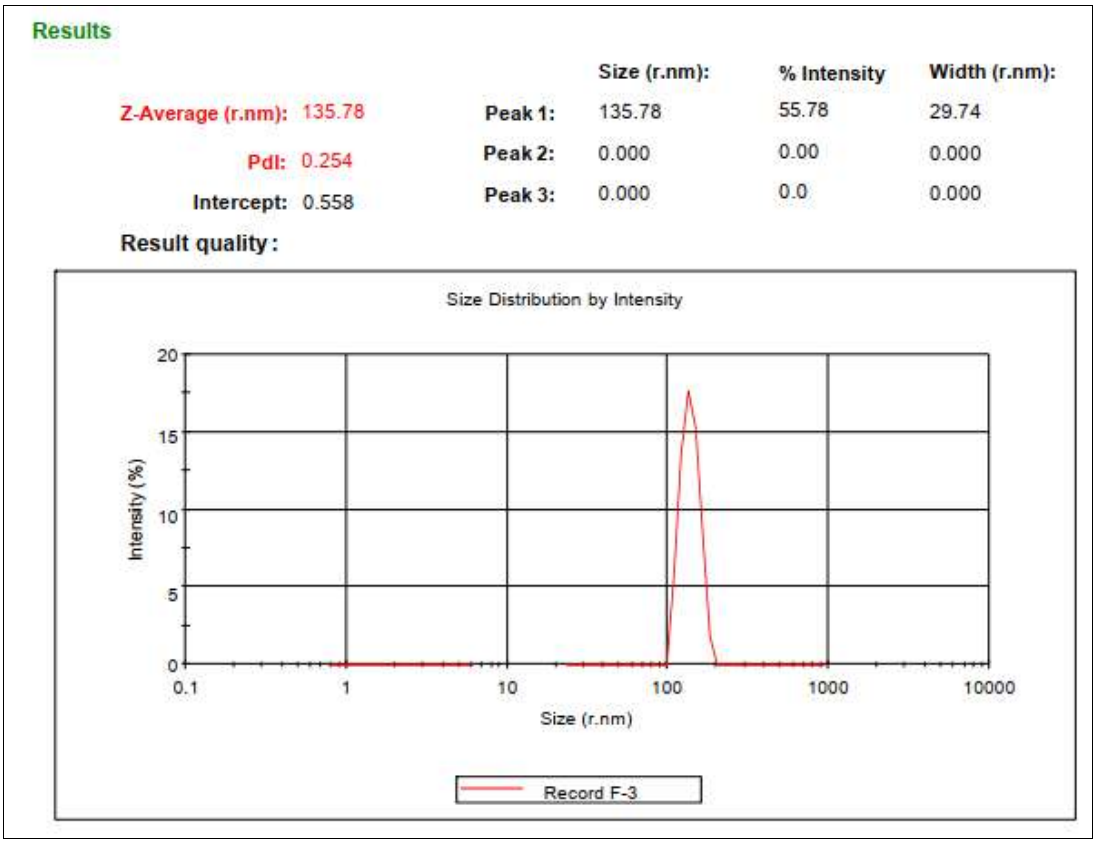

Figure 1: Particle size data of chitosan microspheres (F3)

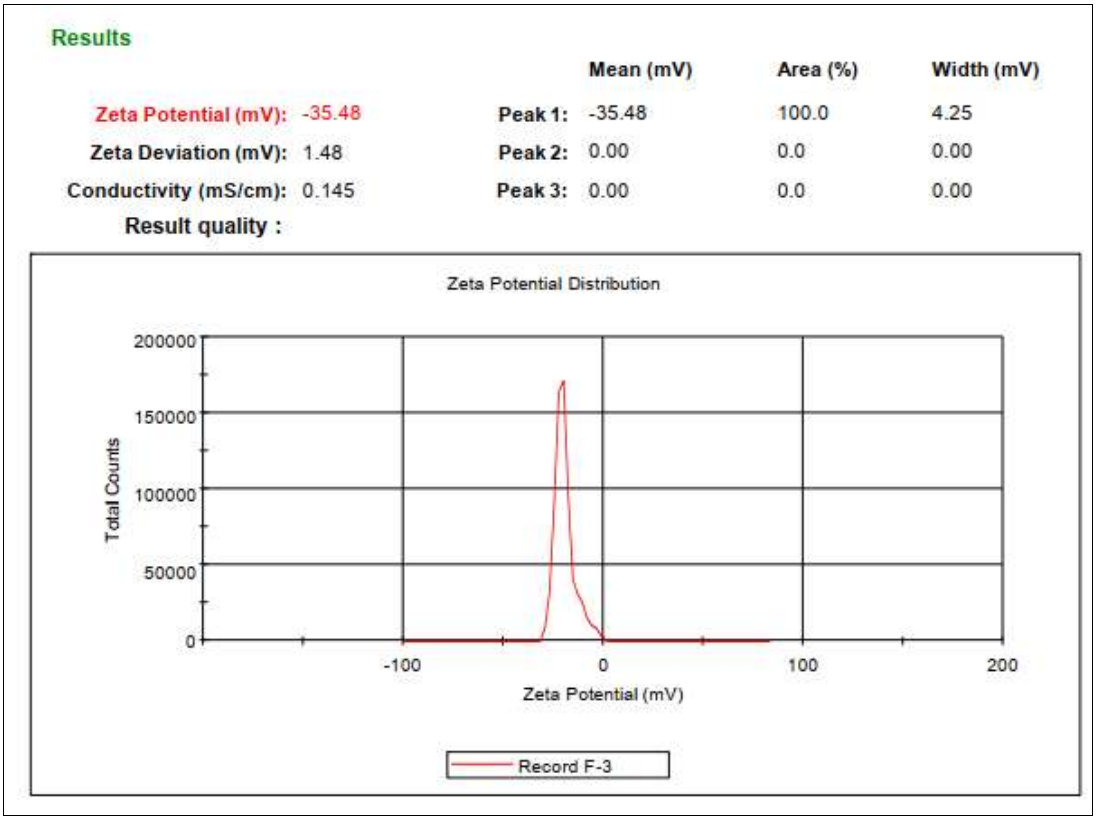

Figure 2: Zeta potential data of chitosan microspheres (F3)

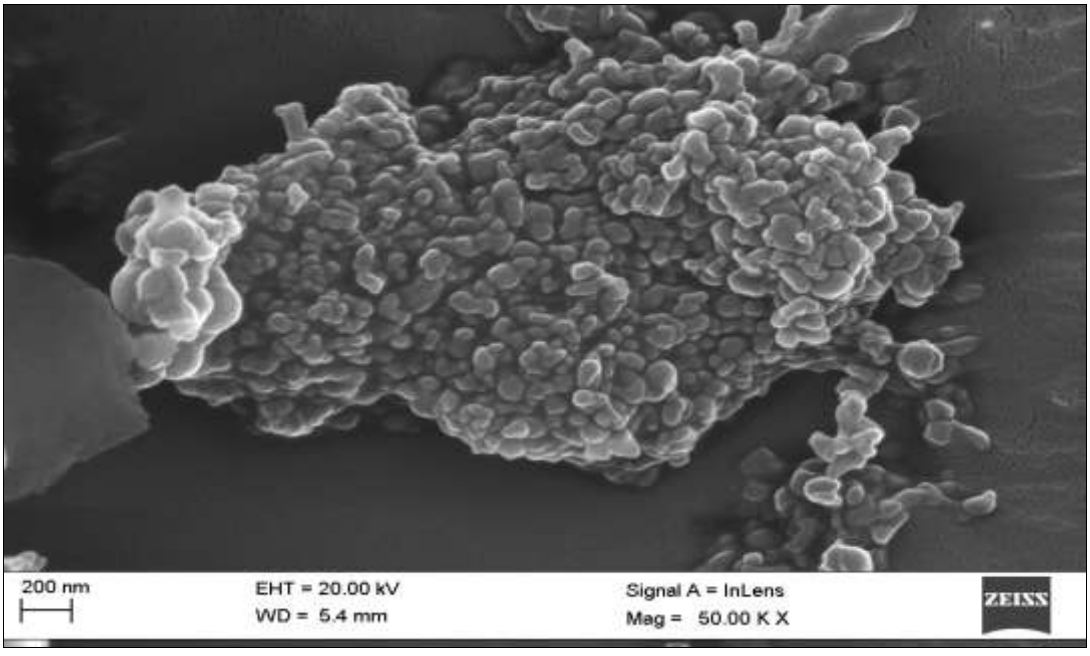

Figure 3: Scanning Electronic Microscopy of optimized formulation (F3)

Table 4: Cumulative % drug release of Olsalazine 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	11.58	1.15
2		2	25.65	2.65
3	SGF+SIF(pH 4.5)	3	30.65	4.48
4		4	42.25	5.85
5		5	50.65	11.65
6	SIF (pH 6.8)	6	65.45	19.98
7		7	71.15	28.89
8	SIF (pH 7.4)	8	76.65	47.78
9		9	87.74	59.98
10		10	90.25	69.98
11		12	98.87	88.87

Table 5: Regression Analysis Data of microspheres Formulation

FORMULATION	ZERO ORDER	FIRST ORDER	HIGUCHI PLOT	PAPPAS PLOT
F3 (R^2)	0.927	0.851	0.831	0.694

Conclusion

Microspheres loaded Olsalazine have been prepared by easy emulsification method followed by cross-linking method. The variables such as drug: polymer ratio and concentration of polymers 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 probable to treat colonic diseases, as a future scope.

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