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COLON TARGETED CHITOSAN MICROSPHERES CONTAINING SULFASALAZINE: PREPARATION AND EVALUATION

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ABSTRACT

Oral colon-targeted microsphere based drug delivery system containing sulfasalazine was prepared, optimized and characterized. The microspheres were successfully prepared by simple emulsification phase-separation technique followed by crosslinking. The formulations were optimized on the basis of drug: polymer ratio, stirring speed, concentration of glutraldehyde. The prepared microspheres were characterized on the basis of morphology, entrapment efficiency,particle size and in-vitro release.

Keywords: Microspheres, colon-targeted drug delivery systems, double-emulsion solvent diffusion method, chitosan, salfasalzine

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INTRODUCTION

Oral colon-targeted drug delivery systems have recently gained recognition for efficient delivery of therapeutic agent for both local as well as systemic action for the treatment of various colonic inflammatory diseases. The successful targeted delivery of drugs to the colon via the gastrointestinal tract requires the protection of a drug degradation and release in the stomach and small intestine and ensures immediate controlled release in the proximal colon [1,2].

Microsphere based drug delivery systems are known to increase the life span of active pharmaceutical ingredient (API) and also involved in controlled release of API. Small particle size of microspheres with large surface area attributed for controlled release of insoluble drugs [3, 4]. Thus microspheres targeted to colon would be a promising for both local and systemic drug delivery. The prepared microspheres may be advantageous in term of reduced dose frequency, improved patient compliances, reduced side effects, high drug loading, and improves bioavailability [5]. Sulfasalazine (SLZ) is the anti-inflammatory drugs used to treat various inflammatory bowel diseases such as ulcerative colitis, and Crohn's disease due to induction of T-lymphocyte apoptosis modulates inflammatory mediators. It is poorly absorbed drug with approximately 5-19 hr elimination half-life [6]. Sulfasalazine is a derivative of mesalazine and also a prodrug of 5 aminosalicylic acid that is covalently linked to the antibiotic sulfapyridine by an azo bond. The objective of 54 present research works is to prepare, optimize and characterize the mucoadhesive microspheres for enhanced delivery of active ingredients [7-9].

MATERIALS AND METHODS

Materials

Sulfasalazine (SLZ) was received as a gift sample from Syntho Pharmaceuticals, Lucknow, India. Chitosan, light liquid paraffin, heavy liquid paraffin, Span 85, lsoproyl alcohol and glutraldehyde were procured from Himedia, Mumbai, India. All other chemicals, reagents and solvents used were of analytical grade.

Preparation of Sulfasalazine Microspheres

The sulfasalazine loaded microspheres were prepared by simple emulsification method followed by cross-linking method. Chitosan solution was prepared by dissolving the 100 mg of chitosan 1% v/v acetic acid (50 ml). The sulfasalazine (100 mg) was added to the disperse phase (chitosan

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solution). The drug-chitosan solution was extruded through a syringe (No. 20) in liquid paraffin (100 ml, heavy and light, 1 : 1 ratio) containing Span 85 (0.5%), and it was stirred at 1500 rpm using mechanical shaker. After 15 minutes, crosslinking agent (v/v aqueous solution) was added and stirring was continued for next 3 hours. The obtained microsphere were filtered and washed with isopropyl alcohol to remove traces of oil. They were finally washed with water to remove excess of crosslinking agent. The microspheres were then dried at 25°C and 60% relative humidity for 24 hrs[10].

Optimization of SLZ Microspheres

The SLZ microspheres were optimized by preparing six formulations (Table 1) using different variables such as drug: polymer ration, stirring speed, volume of gluteraldehyde. The resultant particle size, entrapment efficiency and drug release studies were considered for optimization process.

Table 1: Optimization of SLZ Microspheres

FormulationCode	Variables		
	(Drug: polymer)	(stirring speed) rpm	(Vol. of gluteraldehyde) (v/v)
SLZ-1	(1:1)	(500)	(0.5)
SLZ-2	(1:1)	(1000)	(1.0)
SLZ-3	(1:1)	(1500)	(1.5)
SLZ-4	(1:2)	(500)	(1.0)
SLZ-5	(1:2)	(1000)	(1.5)
SLZ-6	(1:2)	(1500)	(0.5)

Characterization of Microspheres

Morphological Characterization of Microspheres

Scanning electron microscopy is the very adequate method for the investigation of surface morphology of the prepared microspheres. The microsphere samples were prepared by smattering the powder on a double-sided adhesive tape stuck to an aluminum stub. The coating of gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in high-

vacuum evaporator. The coated 55 samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (LEO-430, Cambridge, UK).

Particle Size Analysis

The particle size was determined by microscopic method. For each batch of the microsphere, 100 particles were randomly selected using an optical microscope fitted with a camera (Yoko CDD camera, Taiwan) and Medical Pro software (Version 3.0).

Determination of Encapsulation Efficiency

Weighed amount of microspheres was triturated with 100 ml of phosphate buffer (pH 6.8). The resulting mixture was stirred by magnetic stirrer for 2h. The solution was filtered through a membrane filter (0.45 mm pore size). 1 ml of the filtrate was suitably diluted using phosphate buffer (pH 6.8) and analyzed spectrophotometrically at 359 nm using UV-1700 Pharmaspec, Shimadzu UV- Visible spectrophotometer.

The EE was calculated using the formula.

$$\% \text{ EE} = \frac{\text{Initial amount of drug in NPs} - \text{free drug}}{\text{Initial amount of drug in NPs}} \times 100$$

***In-vitro* drug release study**

A weighed quantity of the microspheres was suspended in 200 ml of phosphate buffer pH 6.8 for 24 hrs using United States Pharmacopoeia basket-type dissolution rate test apparatus. Sample solution (5 ml) was withdrawn at predetermined time intervals and filtered through whatman filter paper. The samples were diluted suitably and analyzed spectrophotometrically with UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu) at 359 nm. % drug release for the batch 5 (SLZ-5).

Stability studies

Six batches of optimized formulation SLZ-5 were stored in amber colored screw capped glass vials in stability chamber at 40±1°C and 75%±5 relative humidity, room temperature and 4±0.5°C (refrigerator) for 3 months. Samples were analyzed for physical appearance, residual

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drug content after a period of 0, 7, 15, 30, 60 and 90 days. Initial drug content was taken as 100 % for each formulation.

Results and Discussion

Microspheres of SLZ have been successfully prepared using by simple emulsification methodfollowed by cross-linking method due to high entrapment efficiency. The various variables (drug: polymer ratio, stirring speed, concentration of crosslinking agent play an important role in the formulation of microspheres and their characteristics.

Morphological characterization of microspheres

The surface topography of the microspheres was investigated by SEM (LEO-430, Cambridge, U. K). Microparticles containing sulfasalazine were small and uniform in size with surface cross-linked and almost spherical and free flowing (Figure 1).

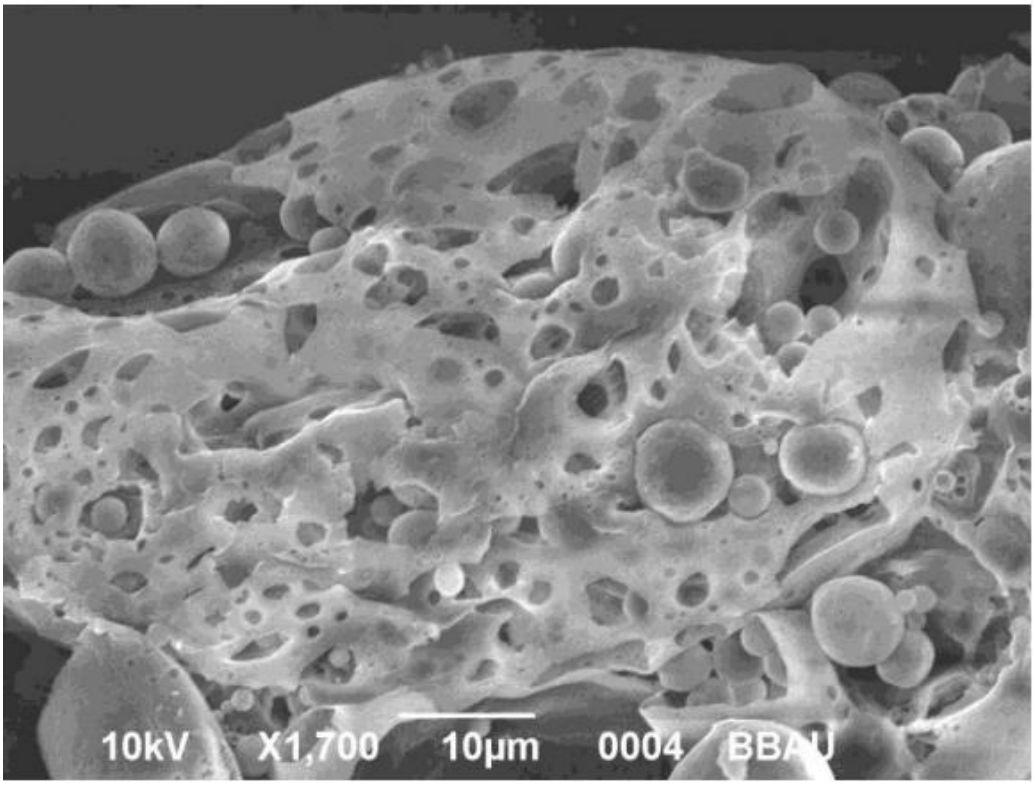

Fig 1: SEM photomicrograph of Chitosan microspheres of SLZ-6

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Particle size analysis

The particle size and size distribution of the 100 randomly selected microspheres were determined using an optical microscope fitted with a camera (Yoko CDD camera, Taiwan) and Medical Pro software (Version 3.0). The particle size of optimized batch (SLZ 5) was found to be 135 µm as shown in Table 2.

Table 2: Experimental data (Optimization of particle size, µm)

Formulation	Observed response value of particle size (µm			Mean (µm)
SLZ-1	360	372	385	372.33
SLZ-2	257	255	270	294
SLZ-3	275	267	245	262.33
SLZ-4	167	162	163	164
SLZ-5	135	131	139	135
SLZ-6	171	167	152	163.33

Drug Entrapment Efficiency

The entrapment efficiency of optimized batch (SLZ-5) was found to be 78.26±0.87%). Entrapment efficiency of microparticles depends on drug: polymer ration, stirring speed and concentration of crosslinking agent (gluteraldehyde).

Table 3: Experimental data and (Optimization of entrapment efficiency, %)

Formulation	Observed response value of encapsulation efficiency (%)			Mean (%)
SLZ-1	45.16	42.17	43.11	43.48
SLZ-2	50.00	51.22	55.00	52.07
SLZ-3	61.09	62.11	67.55	63.58
SLZ-4	70.00	69.15	73.23	70.79
SLZ-5	79.11	78.26	78.27	78.54
SLZ-6	62.00	65.23	67.12	64.78

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***In-vitro* Drug Release Study**

The *in vitro* drug release studies were performed in simulated colonic fluid (pH 6.8). The amount of the drug released from the formulation in dissolution medium without rat caecal contents was found to be (Fig. 2) only 57.55± 0.19 of SLZ-5 respectively.

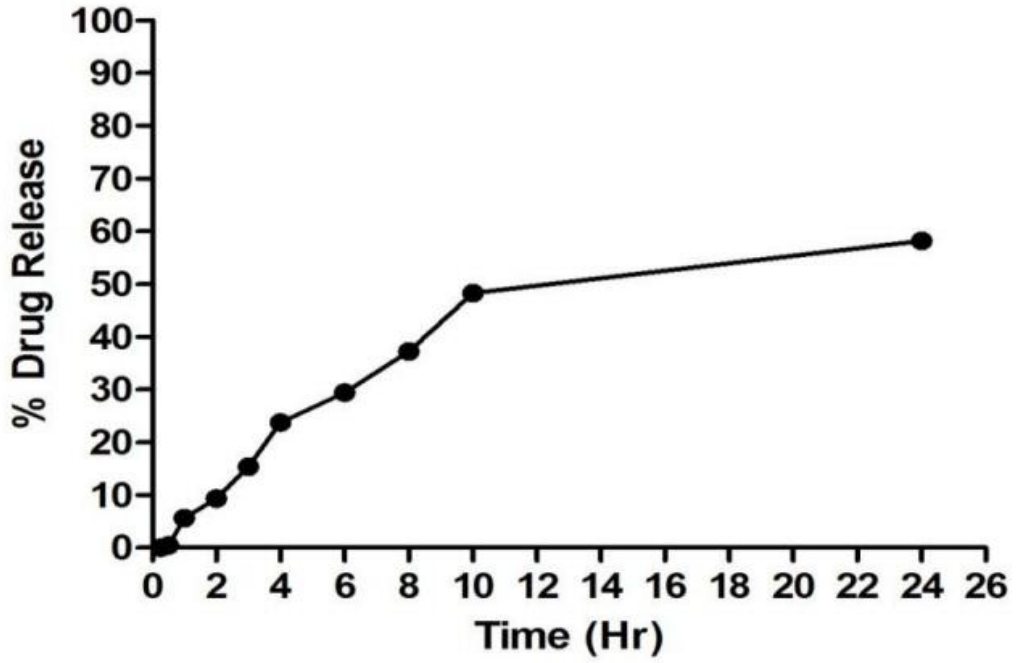

Fig 2: *In-vitro* release profile of optimized batch (SLZ-5) microspheres in simulated colonic fluid (pH 6.8)

Stability studies

The dried SLZ loaded microspheres (SLZ-5) exhibit no considerable difference in residual drugcontent presented in Table 4. The stability result suggested that microspheres were stable upto 90 days at 4°C and 25°C.

Table 4: Stability data for optimized formulation (SLZ-5)

S. No.	Sampling interval (days) %	Residual Drug Content Mean ± S.D. (n=3)		
		4±0.5 °C	Room temp. 40±0.5 °C	75%±5 RH
1	0	100	100	100
2	7	99.68±0.12	99.76±0.20	99.16± 0.15

3	15	99.45±0.06	98.92±0.08	98.45± 0.16
4	30	99.10±0.11	98.65±0.06	97.28± 0.17
5	60	98.45±0.09	98.78±0.05	96.56± 0.19
6	90	98.05±0.07	96.32±0.10	95.10± 0.27

CONCLUSION

Microspheres loaded SLZ have been prepared by simple emulsification method followed by cross-linking method. The variables such as drug: polymer ratio, stirring speed and concentration of glutraldehyde were optimized on the basis of particle size, entrapment efficiency. The prepared microspheres were stable, spherical particles and exhibited favorable release profiles in simulated colonic fluid. 58 However, further evaluation of these carriers can be performed for their potential to treat colonic diseases, as a future scope.

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