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Research Article

Formation development and evaluation of microsphere of sulfasalazine for the treatment inflammatory bowel diseases

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Abstract

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 was prepared, optimized and characterized. The microspheres were effectively prepared by simple emulsification phase-separation technique followed by cross-linking. The formulations were optimized on the basis of drug: polymer ratio, stirring speed, concentration of glutaraldehyde. The prepared microspheres were characterized on the basis of morphology, entrapment efficiency, particle size and *in-vitro* release.

Keywords: Microspheres, Sulfasalazine, Inflammatory bowel disease, Colon-targeted, Chitosan

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INTRODUCTION

Pharmaceutical creation and investigate are progressively more focusing on delivery systems which increase attractive therapeutic objectives while diminishing side effects. Oral drug delivery system represents one of the border regions of drug delivery systems. Such a dosage form supervises widespread concern which exists in region of cost-efficient treatment, patient compliance, most favorable drug delivery and bioavailability[1]. The last two decades there has been a remarkable improvement in the field of novel drug delivery systems. Carrier skill presents a smart approach for drug delivery by pairing the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc, which adjusts the release and absorption traits of the drug[2]. 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[3]. Microspheres are typically free powders consisting of proteins or synthetic polymers that are biodegradable in nature and preferably having a particle size less than 200µm[4]. 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[5-7]. Colon-targeted drug delivery has been the center of many studies in current years due to its potential to progress treatment of local diseases affecting the colon, while minimizing systemic side effects. A number of instances of disease states which impact the colon include CD, UC and irritable bowel syndrome (IBS) [8]. A number of the regularly used drugs for the treatment of this illness comprise hydrocortisone, metronidazole, sulfasalazine, dexamethasone, prednisolone and others[9]. The delivery of these drugs purposely to the colon without being absorbed first in the upper (GI) tract allows for an elevated concentration of the drug to arrive at the colon with negligible systemic absorption[10]. The colonic substances have a longer retention time (up to 5 days), and the colonic mucosa is known to make easy the absorption of numerous drugs, making this organ an perfect site for drug delivery[10,11]. Sulfasalazine (SLZ) is the anti-inflammatory drugs used to treat various IBD such as UC and CD due to induction of T-lymphocyte apoptosis modulates inflammatory mediators. It is poorly absorbed drug with approximately 5-19 hr elimination half-life. Absolute bioavailability of SLZ is <15% when administered orally shown by *in vivo*. SLZ 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[12,13]. The reason of the current study was to prepare, optimize and evaluate the colon-targeted microspheres of SLZ for the treatment and management of IBD.

MATERIAL AND METHODS

Reagents and chemicals

Sulfasalazine was kindly provided as a gift sample from Syntho Pharmaceuticals, Lucknow, India. Chitosan, light

liquid paraffin, heavy liquid paraffin, Span 85, lsoproyl alcohol and glutaraldehyde was purchased from Hi-Media laboratories Mumbai, India. Double distilled water was prepared freshly and used whenever required. All other ingredients and chemicals used were of analytical grade.

Preformulation studies

Physical characteristics

By visual examination, the drug was identified for physical characters like colour, texture, odour etc.

Solubility

Solubility of the drug was determined by taking some quantity of drug (about 1-2 mg) in the test tube separately and added the 5 ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, chloroform and 7.4 pH buffer) Shake vigorously and kept for some time. Note the solubility of the drug in various solvents (at RT).

Melting point determination

Melting point of drug was indomitable by Open capillary method.

Determination of partition coefficient

50 mg of drug was taken in 3 separating funnels. The separating funnels were shaken for 2 hrs in a wrist action shaker for equilibration. 2 phases were alienated and the quantity of the drug in aqueous phase was analyzed spectrophotometrically. The partition coefficient of the drug in phases was calculated by using formula:

K_{PC} = Concentration of Drug in Oil Phase/ Concentration of Drug in Water Phase

Determination of λ_{max} of SLZ

Accurately weighed 10 mg of drug was dissolved in 10 ml of phosphate buffer pH 6.8 solutions in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with phosphate buffer pH 6.8 solution. Prepare suitable dilution to make it to a concentration range of 2-20µg/ml. The spectrum of this solution was run in 400-800 nm range in U.V. spectrophotometer (ShimadzuUV-1600, Japan). A graph of concentration Vs absorbance was plotted.

FTIR spectroscopy

Identification of SLZ was done by FTIR spectroscopy with respect to marker compound. Sulfasalazine was obtained as white to brownish powder. It was identified from the result of IR spectrum as per specification. FTIR spectra recorded on KBr disk method using Brukers Alpha Spectrophotometer with IR solution software. Sample powder was systematically mixed by triturating with KBr in a glass mortar with pestle and compressed into disks in a hydraulic press. FTIR spectra of all the samples were recorded over a spectral region from 4700 to 400 cm-1 using 20 scans with 4 cm-1 resolution.

Preparation of SLZ microspheres

The SLZ loaded microspheres were prepared by easy 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 SLZ (100 mg) was added to the disperse phase (chitosan 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, cross linking 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 eliminate traces of oil. They were finally washed with water to eliminate excess of cross linking agent. The microspheres were then dried at 25°C and 60% relative humidity for 24 hrs[14].

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 glutaraldehyde. The resultant particle size, entrapment efficiency and drug release studies were considered for optimization process.

Table 1: Optimization of SLZ microspheres

Formulation Code	Variables		
	Drug: polymer	Stirring speed (rpm)	Vol. of glutaraldehyde (v/v)
F-1	1:1	500	0.5
F-2	1:1	1000	1.0
F-3	1:1	1500	1.5
F-4	1:2	500	1.0
F-5	1:2	1000	1.5
F-6	1:2	1500	0.5

Characterization of microspheres

Percentage yield

The prepared microspheres with a size range of 200-300nm were collected and weighed from dissimilar formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres.

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

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 were 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 Shimadzu UV-Visible spectrophotometer (UV-1600). 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 (5ml) was withdrawn at predetermined time intervals and filtered through whatman filter paper. The samples were diluted suitably and analyzed spectrophotometrically with UV-Visible spectrophotometer (ShimadzuUV-1600, Japan) at 359 nm.

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 samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (LEO-430).

Stability studies

Six batches of optimized formulation F-5 were stored in amber colored screw capped glass vials in stability chamber at 40±1^{0}C and 75%±5 relative humidity, room temperature and 4±0.51^{0}C refrigerator) for 3 months. Samples were analyzed for physical appearance, residual 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

λ_{max} of SLZ was found to be 359 nm by using U.V. spectrophotometer (Shimadzu-1600, Japan) in linearity range 2-20µg/ml Fig.1 Identification of SLZ was done by FTIR spectroscopy with admiration to marker compound. It was identified from the consequence of IR spectrum as per specification Fig.2.The melting point and partition coefficient of SLZ was found to be 240-242^{o}C and 2.25 respectively. It is very slightly soluble in ethanol; practically insoluble in diethyl ether, chloroform, and benzene; soluble in aqueous solution of alkali hydroxides; practically insoluble in water. Microspheres of SLZ have been effectively prepared using by easy emulsification method followed by cross-linking method due to high entrapment efficiency. A variety of variables (drug: polymer ratio, stirring speed, concentration of cross-linking agent) play an significant role in the formulation of microspheres and their characteristics. Percentage yield of dissimilar formulation was determined by weighing the microspheres after drying.The percentage yield of dissimilar formulation was in range of 75.65- 81.25%. The drug entrapment efficacies of dissimilar formulations were in range of 43.47-79.54% w/w. This is because of the mucoadhesion characteristics of chitosan that could make easy the diffusion of part of entrapped drug to surrounding medium during preparation of SLZ microspheres Table 2. On the basis of the utmost percentage yield and drug entrapment was establish to be formulation F-5 in mucoadhesive microspheres so formulation F-5 was further studies. The consequences of measurement of mean particle size of optimized formulation F-5 of mucoadhesive microsphere was found 136.43µm as shown in Table 3. Shape and surface characteristic of SLZ microspheres examine by Scanning Electronic Microscopy analysis. Surface morphology of formulation examines at two different magnifications 55X which illustrate the smooth surface of microspheres Fig. 3. The *in vitro* drug release studies were performed in simulated colonic fluid (pH 6.8). The quantity of the drug released from the formulation in dissolution medium without rat caecal contents was found to be only 99.78 of F-5. According to ICH guidelines, 3 months accelerated stability study at 40±2°C and 75±5% RH, room temperature and 4±0.51^{0}C refrigerator) optimized formulations (F-5) was carried out. It showed slight change over time for parameters like appearance and drug content, No noteworthy difference observed in the drug content between initial and formulations stored at 4°C and room temperature for 3 months.

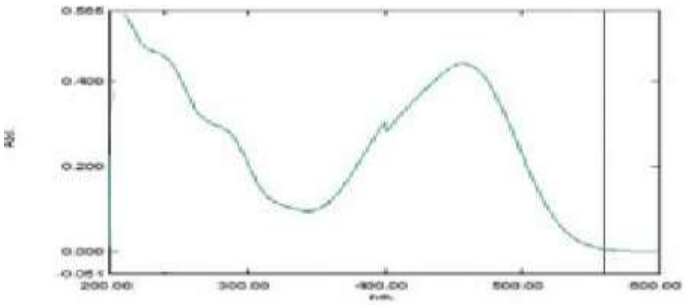

Figure 1 UV spectra of SLZ in phosphate buffer (pH 6.8)

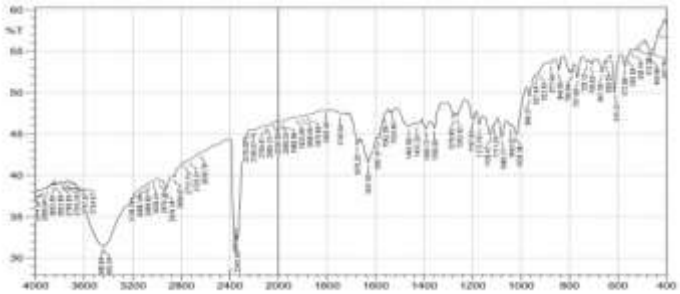

Figure 2 FT-IR spectrum of pure drug (SLZ)

Table 2: Percentage yield and drug entrapment for different formulation

Formulation	Percentage Yield	Drug entrapment (% w/w) of prepared microsphere
F_1	78.98±0.25	43.47±0.25
F_2	79.98±0.12	51.07±0.56
F_3	76.56±0.36	62.67±0.47
F_4	76.56±0.25	71.23±0.58
F_5	81.25±0.14	79.54±0.65
F_6	75.65±0.56	65.76±0.84

Table 3: Experimental data (Optimization of particle size, µm)

Formulation	Particle size (µm)
F_1	371.23
F_2	293.12
F_3	259.32
F_4	167.65
F_5	136.43
F_6	163.56

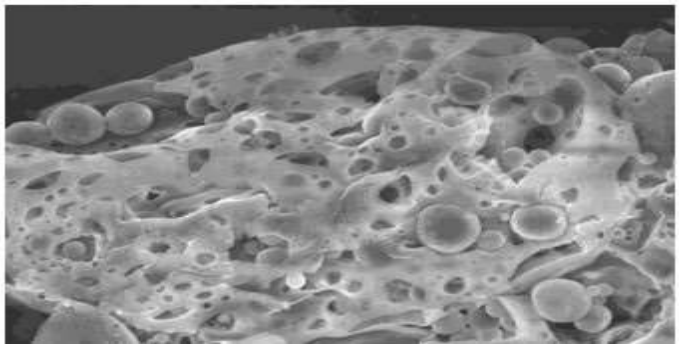

Figure 3 SEM image of optimized mucoadhesive formulation F-5

Table 4: Release study data of formulation F1-F6

Time	% of Drug Release					
(hr)	F1	F2	F3	F4	F5	F6
0.5	43.25	40.25	38.65	20.21	26.65	15.56
1	56.65	50.32	48.98	36.65	39.98	28.89
2	78.89	68.98	65.45	40.54	45.65	38.14
4	98.21	85.56	80.25	50.25	59.98	45.65
6		99.89	89.98	68.98	72.45	65.54
8			99.74	75.56	85.56	72.25
10	-	-		82.45	94.56	79.98
12	-	-		85.65	99.78	83.21

CONCLUSION

Microspheres loaded SLZ have been prepared by easy emulsification method followed by cross-linking method. The variables such as drug: polymer ratio, stirring speed 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 probable to treat colonic diseases, as a future scope.

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