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Preparation and characterization of azathioprine microspheres for colon specific delivery

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ABSTRACT

The objective of the present study is to develop colon targeted drug delivery system using dextrin (polysaccharide) as a carrier for Azathioprine. Microspheres containing azathioprine, dextrin and various excipients were prepared by solvent evaporation technique. The prepared microsphere were evaluated by different methods parameters like particle size, drug entrapment efficiency, percentage yield, shape and surface morphology and in vitro drug release study. Drug release profile was evaluated in simulated gastric, intestinal fluid and simulated colonic fluid. Best formulation was decided on the basis drug release profile in simulated gastric, intestinal fluid and simulated colonic fluid. In dextrin based microspheres, dextrin as a carrier was found to be suitable for targeting of Azathioprine for local action in the site of colon. Dextrin microspheres released 95-99% of azathioprine in simulated colonic fluid with 4% human fecal matter solution. The results of in-vitro studies of the azathioprine microspheres indicate that for colon targeting dextrin are suitable carriers to deliver the drug specifically in the colonic region. Dextrin based azathoprine microspheres showed no significance change in particle size and % residual upon storage at 5 ± 3ºC, 25 ± 2ºC/60 ± 5% RH (room temperature) and 40 ± 2ºC/75 ±5%RH humidity for three months.

Keywords: azathioprine, microsphere, dextrin, colon specific drug delivery.

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1. INTRODUCTION

Since from last decade a novel oral colon-specific drug delivery system (CDDS) has been developing as one of the site-specific drug delivery systems. This delivery system, by means of combination of one or more controlled release mechanisms, this system hardly allow to releases drug in the upper part of the gastrointestinal (GI) tract, but rapidly releases drug in the site of colon following oral administration[1, 2, 3]. CDDS is convenient for treating localized colonic diseases, i.e. ulcerative colitis, Crohn's disease and constipation etc., CDDS, also selectively deliver drug to the colon, but not to the upper GI tract [1]. Colon is referred to as the optimal absorption site for protein and polypeptide after oral administration, because of the existence of relatively low proteolytic enzyme activities and quite long transit time in the colon. CDDS would be advantageous when a delay in absorption is desirable from a therapeutically point of view, as for the treatment of diseases that have peak symptoms in the early morning and that exhibit circadian rhythms, such as nocturnal asthma, angina and rheumatoid arthritis [1, 2, 4].

There were currently a few strategies to achieve colonic specificity, such as use of pH sensitive polymers and pressure-controlled CDDS. The aim of this study was to explore the feasibility of the colonic microorganism to develop CDDS by using azathioprine and budesonide as a model drugs [1, 5].

Polysaccharides, the monosaccharide polymer retains their integrity because they are resistant to the digestive action of gastrointestinal enzymes of stomach. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine but once they reach in the colon, they are acted upon by the bacterial polysaccharides and results in the degradation of the matrices. A large number of polysaccharides such as amylose, guar gum, pectin, chitosan, inulin, cyclodextrins, chondroitin sulphate, dextrans, dextrin and locust bean gum have been investigated for their use in colon targeted drug delivery systems. The most important fact in the development of polysaccharide derivatives for colon targeted drug delivery is the selection of a suitable biodegradable polysaccharide. As these polysaccharides are usually soluble in water, they must be made water insoluble by cross linking or hydrophobic derivatisation, very important is an optimal proportional of the hydrophobic and hydrophilic parts respectively and the number of free hydroxyl groups in the polymeric molecule. The objective of the present study is to develop colon targeted drug delivery

system by using dextrin as a carrier for Azathoprine. CDDS is also selectively delivered drug to colon but not to the upper tract [6, 7].

2. MATERIAL AND METHODS

Azathioprine was obtained from Sun Pharmaceuticals Limited, Mumbai. Dextrin and Span 80 was purchased from Sigma Aldrich Pvt Ltd. All other ingredients, solvents used for assay were of analytical grade.

Method

The azathioprine microspheres were prepared by solvent evaporation method. The drug (azathioprine) and dextrin (1%, 5%, 10% & 20% w/v) were dissolved in dichloromethane. This solution was dispersed in 100 ml of liquid paraffin light containing various concentration of Span 80 in a 250 ml beaker. The dispersion was stirred at 200 rpm for 30 min. After the stirring time, microspheres were centrifuged, washed several times with n-hexane, ether and finally with acetone. The microspheres were dried at 50°C and stored in desiccator [8]. Than optimization done on the basis of drug concentration, stirring time, surfactant concentration, temperature, and polymer concentration shown in table 1. Total 19 batches were prepared for azathioprine microspheres. In which 4 formulations were prepared with different drug polymer ratio. These 4 formulation of azathioprine microspheres further evaluated.

Table 1: Optimization of Azathioprine microspheres

Formulation code	Drug in Mg	Span 80 (%w/w)	Stirring speed (rpm)	Temperature	Dextrin (%w/v)	Mean size (µm)	% drug entrapment efficiency
F1 A	25	0.5	400	37 º	5	83±2.8	94.2±1.4
F2 A	50	-	-	-	-	85±1.9	83.5±1.8
F3 A	75	-	-	-	-	87±1.8	78.8±1.2
F4 A	100	-	-	-	-	88±1.2	74.3±2.8
F5 A	25	0.5	400	37 º	5	75.8±1.2	92.8±1.4
F6 A	-	0.75	-	-	-	73.9±0.9	74.8±1.2
F7 A	-	1.0	-	-	-	71.2±1.2	72.5±2.6
F8 A	-	1.25	-	-	-	70.2±1.6	68.7±2.2
F9 A	25	0.5	200	37 º	5	80.2±2.2	92.6±1.4
F10 A	-	-	300	-	-	76.4±1.8	84.3±2.6
F11 A	-	-	400	-	-	72.3±1.6	81.6±2.6
F12 A	-	-	500	-	-	66.2±1.2	79.6±2.4
F13A	25	0.5	200	25 º	5	71.3±2.2	83.8±1.6
F14 A	-	-	-	37 º	-	76.2±1.8	92.6±1.6
F 15 A	-	-	-	45 º	-	78.2±1.8	78.2±1.6
F16 A	25	0.5	200	37 º	1	75.4±1.6	76.8±1.2
F17 A	-	-	-	-	5	82.8±1.2	83.5±1.8
F18 A	**-**	**-**	**-**	**-**	**10**	**85.4±0.9**	**95.2±1.4**
F 19 A	-	-	-	-	20	78.5±1.2	83.5±1.8

Evaluation Parameters

The prepared microspheres of Azathioprine drug were evaluated for particle size, drug entrapment efficiency, percent yield, invitro release studies and stability studies.

Particle size analysis

The particle size of microspheres was determined using optical microscopy method. Particle size of all the batches of azathioprine microspheres and budesonide microspheres sample was measured with an optical micrometre fitted with a calibrated eye piece. Approximately 200 particles were counted for particle size using a calibrated optical microscope. All readings are average of three trials ± SD [9].

Drug entrapment efficiency

Drug entrapment efficiency Drug entrapment efficiency of Azathioprine microspheres was performed by accurately weighing 100 mg of drug equivalent microspheres and suspended in 100 ml of 7.4 pH phosphate buffer and it was kept on a side for 24 hours. Then, it was stirred for 15 mints and filtered. After suitable dilution, azathioprine in the filtrate was analyzed spectrophotometrically at 280 nm using U.V. Spectrophotometer [10].

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \text{x } 10$$

Percentage yield

The prepared azathioprine microspheres with a size range of 60-90 µm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the microspheres [11].

$$\% \text{ Yield} = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} \text{x } 10$$

Scanning electron microscopy analysis (SEM)

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan). Sample was fixed on carbon tape and fine gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was set at 3.0 KV during scanning. Microphotographs were taken on different magnification and higher magnification (500X) was used for surface morphology.

In vitro drug release study

Test were carried out for azathioprine microspheres using separate USP apparatus II (paddle) and the medium was Simulated gastric fluid, Simulated intestinal fluid and

simulated colonic fluid, individually. Quantity of dissolution medium was 900 mL. The speed of paddle was 50 rpm and temperature of dissolution medium was 37.5o C. The accurately weighed azathioprine microspheres were placed in the dissolution medium and apparatus was run. At intervals of 2, 5, 8, 12, 16, 20 and 24 hours, 5 mL aliquots were withdrawn and replacement was made each time with 5 mL of fresh dissolution medium [6]. Each 5 mL sample was filtered through Whatman filter paper no. 41 and diluted up to 50 ml with respective dissolution medium. Then absorbance was measured at 280 nm [12].

Stability Study

Stability studies were carried out at5 ± 3ºC, 25 ± 2ºC/60 ± 5% RH (room temperature) and 40 ± 2ºC/75 ±5% RHfor the optimized formulation F18 A and F18 B for 3 months. The samples were withdrawn after predetermined period of 1 month, 2 month and 3 month. The samples were analyzed for its particle size and % residual drugs [13].

Drug Release Kinetics

Zero order release rate kinetics [14]

To study the zero order release kinetics the release rate data are fitted to the following equation F=K0t

Here, F is the fraction of drug release

K0 is the rate constant

T is the release time

First order model

This model has also been used to describe absorption and/elimination of drug, the release of the drug which followed first order kinetic can be expressed by the equation

Log C=log c0-kt/2.303

Where, Co is the initial concentration of drug

K is the first order rate constant

t = is the time

Higuchi release model

To study the higuchi release kinetics, the release rate data was fitted to the following equation

F =KH.t1/2

Where, F is the amount of the drug release

Kh is the release time

t is the release time

Korsmeyer and peppas model

The release rate date were fitted to the following equation,

Mt/M8 =KM.tn Where,

Mt/M8 is the fraction of drug release

KM is the release constant t is the release time.

3. RESULTS AND DISCUSSION

Evaluation parameters of microsphere

Microspheres of azathioprine were prepared successfully by solvent evaporation method. The various parameters in the production of microspheres were evaluated and reported in Table 1. The particle size study was found to be between 75.4 ±1.6 to 85.4 ± 0.9 of F16 A - F19 A. The particle size of microspheres increased with increases in the polymer concentration. The drug entrapment efficiency was found to be range of 76.8 ± 1.2 to 95.2 ± 2.1. Initially the entrapment efficiency microspheres is increased with increasing the polymer concentration but after optimum condition entrapment efficiency is decreased due to less amount of drug available as compare to polymer for entrapment. The Percent yield was found to be between 71.56 ± 1.4 to 84.23 ± 2.5. The shape and surface characteristics were determined by scanning electron microscopy and report shows in Fig 1.

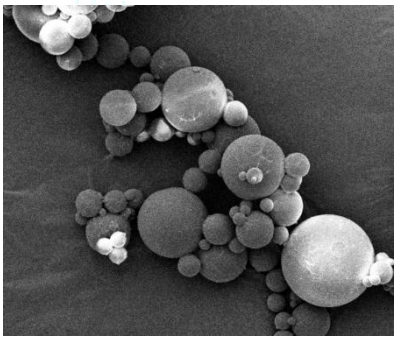

Figure 1: SEM image of optimized formulation F 18 A

Dissolution Studies

All the F16 A – F19 A formulations of azathioprine microspheres are subjected to dissolution studies. Dissolution is carried out in USP type II apparatus at 50 rpm in the volume of 900ml dissolution medium was simulated gastric fluid, simulated intestinal fluid and simulated colonic fluid for 24 hours. The results are shown in Table 2 and Fig.2.

Table 2: Release study of Formulation F16 A- F19 A

Simulated Media (pH)	Time in Hours	F16 A	F17 A	F18 A	F19 A
		Cumulative % Drug release			
SIF 1.2	1	15.42	10.17	4.23	11.40
	2	26.30	18.58	8.96	20.86
SIF 6.8	5	40.22	36.56	12.64	34.70
SCF 7.4	8	60.32	68.84	56.20	62.17
	12	68.46	72.08	68.00	73.32
	16	75.37	89.40	86.80	84.39
	20	83.92	92.82	95.50	90.46
	24	96.70	98.08	99.01	97.20

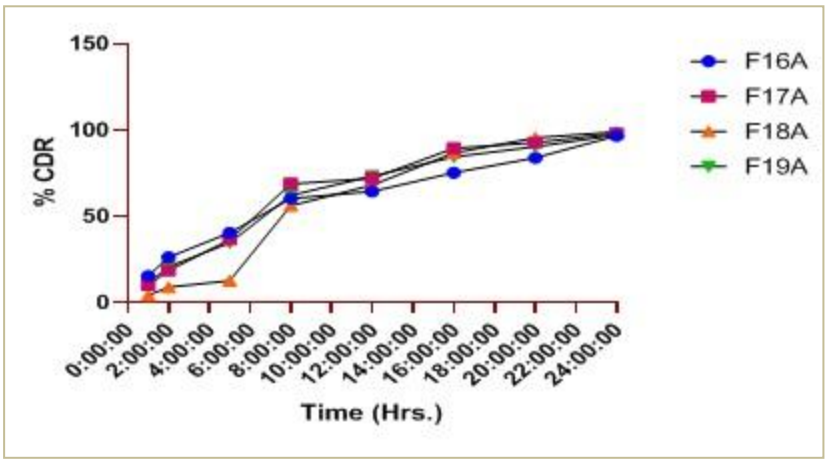

Figure 2: Release study of formulation F16 A - F19 A

Drug release studies shows that F18 A shows good release behavior in colon and restricts release in stomach and intestine as compare to F16 A, F17 A, and F19 A. Because dextrin is degraded by the enzymes presented in the colonic region hence this study confirms that for colon targeting by dextrin can act as good carrier.

Stability study of formulation F 18 A confirms that microsphere are more stable in refrigerator condition at 5 ± 3º and there was no significant change occurs in particle size and % residual drug content of F18 A.

The Fig. 2 indicates F18 formulation shows better drug release when compared with other formulations, and followed by the first order kinetics. The mechanism of release F18 A formulation as shown in Fig 3-6.

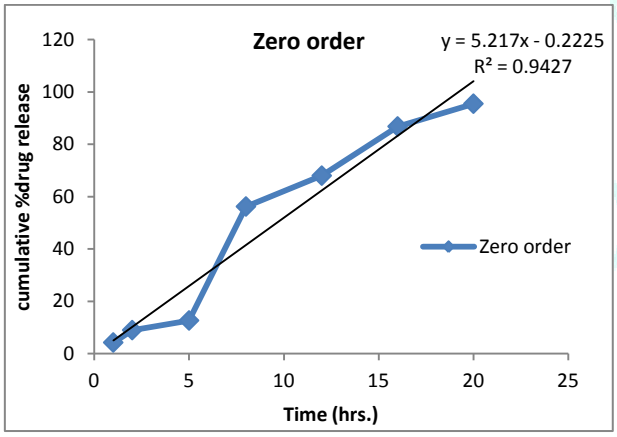

Figure 3: Zero order release kinetics of F18 A

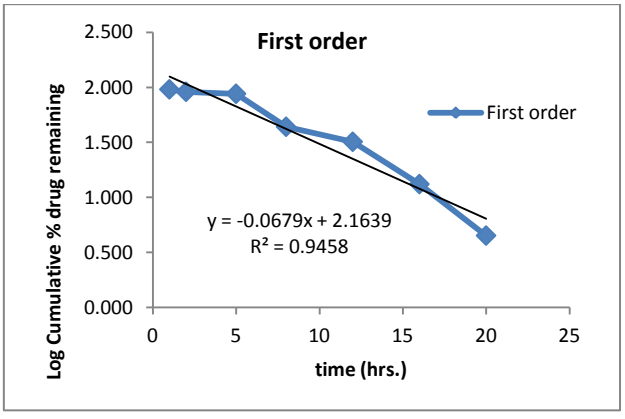

Figure 4: First order release kinetics of F18 A

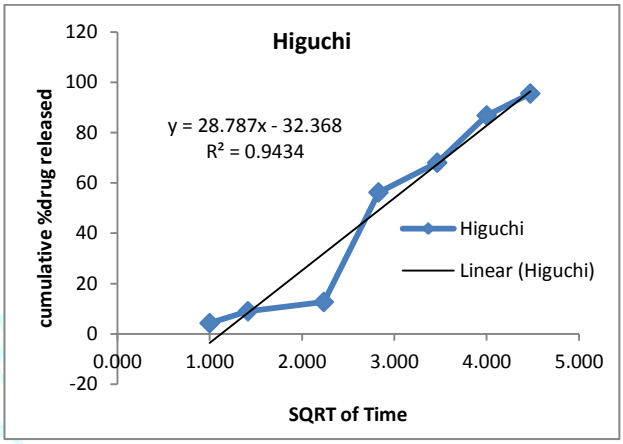

Figure 5: Higuchi order release kinetics of F18 A

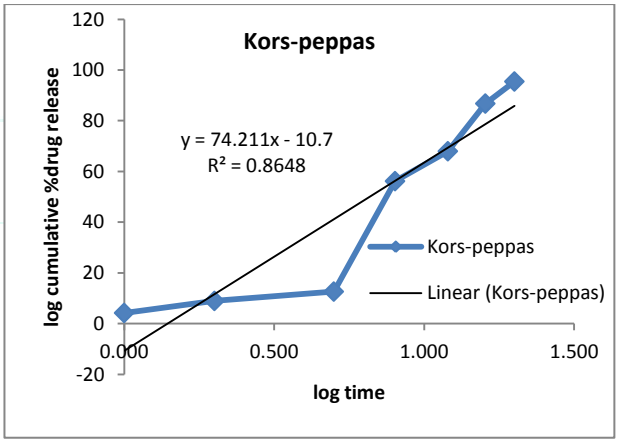

Figure 6: Korsemayer – Peppas release kinetics of F18 A

4. CONCLUSION

Drug release studies showed that Dextrin based azathioprine microspheres shows good release behaviour in colon and restricts release in stomach and intestine. This study confirms that for colon specific targeting, dextrin can act as good carrier which delivers the more amount of drug specifically in colon.

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