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Research Article

FORMULATION DESIGN AND EVALUATION OF GASTRORETENTIVE MUCOADHESIVE MICROSPHERES OF CLARITHROMYCIN

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ABSTRACT

The objective of the present study was to develop gastroretentive mucoadhesive microspheres of clarithromycin to combat *Helicobacter pyroli* infection in ulcer patients. Clarithromycin is used for the treatment of *H. pyroli*. Clarithromycin attains peak plasma concentration in 2 hours after administration. Its dosing schedule is 8 to 12 hour but the level of drug use to drop below its Minimum Inhibitory Concentration (MIC) well before administration of the subsequent dosing. Clarithromycin has low bioavailability i.e. 50-60% due to its first pass metabolism. Retaining the drug at a site above its absorption site i.e. in the stomach would allow the drug to be absorbed effectively from its site of absorption and gives time for healing of peptic ulcer caused by *H. pyroli* infection by increasing time for local action of drug. The microspheres were prepared using Sodium alginate and Hydroxypropyl Methylcellulose as polymer by Ionic Gelation Technique. The microspheres were evaluated for size, shape, entrapment efficiency and drug release. An increase in concentration of Sodium alginate results in increase in Mean particle size of microspheres whereas on increasing concentration of Hydroxypropyl Methylcellulose an increase in mucoadhesive properties and drug release was observed.

Keywords: Mucoadhesive microspheres, Clarithromycin, *H. pyroli*, *in vitro* release.

INTRODUCTION

Clarithromycin is a macrolide antibiotic, used for the treatment of Legionellosis, *Helicobacter pylori*, and lyme disease. In addition, it is also used to treat tonsillitis, acute maxillary sinusitis, acute bacterial exacerbation of chronic bronchitis, pneumonia (especially atypical pneumonias associated with *Chlamydia pneumoniae* or TWAR), skin and skin structure infections. The standard dose of clarithromycin is 250 mg twice daily by oral route[1]. The low bioavailability (50-60%) and short biological half life (3-4 hours) of Clarithromycin favours the development of sustained release microspheres. Gastroretentive dosage forms are drug delivery systems which remain in the stomach for an extended period of time and allow both spatial and time control of drug liberation[2]. Basically gastroretentive systems swells following ingestion and is retained in the stomach for a number of hours, while it continuously releases the incorporated drug at a controlled rate to preferred absorption sites in the upper intestinal tract[3]. Their application can be advantageous in the case of drugs absorbed mainly from the upper part of GIT or are unstable in the alkaline medium of distal intestinal regions[4]. They are also beneficial in the local therapy of the stomach[5]. Therfore, it is expected that if local delivery of Clarithromycin from the gastric lumen into the mucus layer is achieved, the *H. pyroli*

eradication rate can be fastened. Kimura et al. reported that a one hour treatment regimen provided more complete eradication than conventional therapy due to the extended gastric residence times of the antimicrobial agents[6]. Akiyama et al confirmed that mucoadhesive microspheres have the ability to adhere to the stomach walls in rats and thereby remain in the gastrointestinal tract for an extended period of time[7]. Microspheres are an important type of novel drug delivery system and tremendous research is going on to optimize them to a suitable extent so that their use in humans can be implemented[8]. Mucoadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs due to a high surface to volume ratio, an effective contact with the mucus layer, and a specific targeting of drugs to the absorption site[9]. The term mucodhesion is defined as adhesion to the biological surfaces i.e. mucus and/or mucosal surface. Mucoadhesion can be achieved by different mucin-polymer interactions such as wetting and swelling of the polymer to permit intimate contact with the biological tissue/interpenetration of bioadhesive polymer chains and entanglement of polymer and mucin chains/formation of weak chemical bonds/ sufficient polymer mobility to allow spreading/water transport followed by mucosal dehydration[10]. The aim of the study was to design gastroretentive mucoadhesive microspheres to release the drug (Clarithromycin) in stomach for extended period of time to exert local effect on the ulcer and maximize the effect of the drug on the pathogens (*H. pylori*).

MATERIAL AND METHOD

Clarithromycin was obtained as a generous gift sample from Ranbaxy Laboratories, Gurgaon. Hydroxypropyl Methylcellulose (HPMC, having a viscosity of 50 cps in a 2% by wt/vol aqueous solution at 20°C) obtained as a gift samples from M/s Natco Pharma Ltd (Hyderabad, India). Sodium alginate and Groundnut Oil were procured from Central Drug house (New Delhi). All the chemicals were of analytical grade and Purified Water was used throughout the experiment.

EXPERIMENTAL

Preparation of mucoadhesive microspheres

Sodium alginate and Hydroxypropyl Methylcellulose were dispersed in purified water (32 ml) with continuous stirrring to form homogenous polymer dispersion in different ratio as mentioned in Formulation chart (Table No. 1). Clarithromycin was added to polymer dispersion and mixed thoroughly to form a viscous suspension. The stream of smooth viscous suspension was added to groundnut oil in the form of a thin stream. Stirring of the above mixture was done in a beaker placed on magnetic stirrer. Then Calcium Chloride solution (10% w/v) was added slowly while stirring for ionic gelation reaction. The stirring was continued for 15 minutes. The mixture was centrifuged and product was separated.

CHARACTERIZATION & EVALUATION OF MICROSPHERES

Morphology and Particle Size Determination

Microspheres were observed under a light microscope using a calibrated ocular micrometer. About 100-150 microspheres were observed to calculate the average particle size.

a) **Effect of stirring speed on particle size**
   The effect of stirring speed on the particle size was determined at 400 rpm and 800 rpm.

b) **Surface morphology by Scanning Electron Microscopy**
   The external surface morphology of the microspheres was studied by scanning electron microscopy using Philip 505 apparatus.

Drug entrapment efficiency

To determine the entrapment efficiency, the microspheres were washed and lysed using acetone. After 24 hrs the solution was filtered and filtrate was analysed for drug content using Ultraviolet spectroscopy. The drug entrapment efficiency was calculated using the following formula:

$$\text{Drug entrapment Efficiency} = \frac{\text{Amount of drug present in formulation}}{\text{Theoretical amount of drug in formulation}} \times 100$$

Mucoadhesion study

The *in vitro* mucoadhesive test was carried out using small intestine from albino rat[11]. The tissue was washed with normal saline. A segment of intestine was everted using a glass rod. Ligature was placed at both ends of the segment. 150 microspheres were scattered uniformly on the everted sac from 2 cm height. The sac was suspended in a boiling tube

containing normal saline by a wire. The sac was incubated at 37ºC and agitated horizontally. The sac were taken out of the medium after immersion for 0.5, 1, 1.5, 2 and 2.5 hrs, repositioned as before in a similar tube containing fresh saline. The unbound microspheres were counted.

Mucoadhesion =

(No. of microspheres adhered/No. of microspheres applied) x 100

In-vitro drug release

The release profile of microspheres was determined by using USP type I dissolution test apparatus at 50 rpm and 37ºC temperature. Five ml of aliquots were withdrawn at predetermined intervals and filtered. The required dilutions were made with 0.1 N HCl and the solutions were analysed for the drug content by UV spectrophotometer against suitable blank. Equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain sink condition. From this the percentage of drug released was calculated and plotted against function of time to study the pattern of drug release.

RESULT AND DISCUSSION

The mucoadhesive microspheres of Sodium alginate and HPMC were prepared by Ionic Gelation method. The polymer Sodium alginate was used to control the release rate and HPMC as a mucoadhesive polymer. Five formulations ie MM_1, MM_2, MM_3, MM_4 and MM_5 were prepared by varying concentration of Sodium alginate and HPMC to study the effect of polymer concentration on the size, percentage mucoadhesion and drug entrapment efficiency.

Separately, to study the effect of stirring speed on the size of microspheres five batches (SM_1, SM_2, SM_3, SM_4 and SM_5) were prepared at 400 rpm and 800 rpm. The results were compiled in Table No. 2 and were found to be in compliance with the general theory that on increasing the stirring speed, the size of microspheres decreases. The particle size and surface morphology was determined with the help of light microscope (having ocular micrometer) and Scanning Electron Microscope. Spherical microspheres were observed with microscope and the particle size between 45.6-53.7 µm at 400 rpm and 43.3-47.8 µm at 800 rpm.

Surface morphology of the microspheres was examined by scanning electron microscope (SEM) (Philip 505) Fig I. It was observed that the surface morphology of microspheres depends upon the concentration of different polymers used. The formulation having higher concentration of Sodium alginate have rough surfaces while as its concentration decreases and the concentration of HPMC increases, the surface of microspheres starts becoming smoother. The microspheres having higher proportion of Sodium alginate were bigger in size for e.g. microspheres belonging to SM_1 batch were having maximum size among all the batches. Formulation MM_5 showed the least particle size 52.6 because it contains higher proportion of HPMC. The particle size was found to be 59.7, 56.6, 55.1, 53.9 and 52.6 for formulations MM_1, MM_2, MM_3, MM_4 and MM_5 respectively. MM_3 shows the particle size in between MM_5 and MM_1 because it contains equal proportion of Sodium alginate & HPMC. Formulation MM_1 & MM_5 showed entrapment efficiency 63% & 49% respectively, when polymer HPMC & Sodium alginate ratio is 1.5: 0.5 (for MM_1) and 0.5: 1.5 (for MM_5). On increasing the concentration of HPMC polymer, the amount of drug entrapment increases which indicates that HPMC shows good entrapment efficiency than Sodium alginate as given in table 3.

The *in-vitro* wash off test was performed to check the mucoadhesion properties of the microspheres. Formulation MM_5 showed the highest mucoadhesivity 79.6% due to the higher proportion of HPMC and formulation MM_1 showed the lowest mucoadhesivity 61.8% due to higher proportion of sodium alginate resulting in increased surface irregularity. The percent mucoadhesivity was found to be 61.8, 67.3, 69.8, 74.4 and 79.6% for formulation MM_1, MM_2, MM_3, MM_4 and MM_5 respectively.

The release studies show the different release rate of the microspheres prepared with different polymer ratio. The *in-vitro* release study was observed in HCl (pH 1.2) for 8 hrs. It was found that the release rate from all the formulations varied according to the ratio of polymers used in the formulation i.e. 72.7%, 77.1%, 79.5%, 82.5% and 85.7% for the formulation MM_1, MM_2, MM_3, MM_4 and MM_5 respectively (Fig. III). The formulation MM_5 having highest proportion of polymer HPMC showed maximum release, while MM_1 shows the least drug release after 8 hrs due to less

swelling action and irregular surface as compared to MM_5.

CONCLUSION

The present study revealed that both the polymers used in the formulation of microspheres i.e. HPMC and Sodium alginate have a significant effect on the mucoadhesion, drug entrapment efficiency and drug release. HPMC has good entrapment efficiency and good mucoadhesion while sodium alginate was used to control the release rate. After evaluating all the formulations, the formulation MM_5 which had the higher percentage of HPMC showed good entrapment efficiency (63%), mucoadhesion about 79.6% as well as good release profile in 8 hrs. So, MM_5 can be selected as the best formulation among all the formulations.

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Table 1: Formulation Chart

S. No.	Formulation Code	Drug	Sod. Alginate	HPMC
1	MM_1	1	1.50	0.50
2	MM_2	1	1.25	0.75
3	MM_3	1	1.00	1.00
4	MM_4	1	0.75	1.25
5	MM_5	1	0.50	1.50

Table 2: Effect of stirring speed on Particle size

S. No.	Formulation	Mean Particle Size (µm)	
		At stirring speed 400 rpm	At stirring speed 800 rpm
1	SM1	53.7	47.8
2	SM_2	51.6	46.5
3	SM_3	49.9	45.9
4	SM_4	47.3	45.2
5	SM_5	45.6	43.3

Table 3: Particle size, % drug entrapment and % mucoadhesion of different formulations

S. No.	Formulation Code	Particle Size (µm)	% Drug Entrapment	% Muco adhesion
1	MM_1	59.7±1.01	49±1.09	61.8±1.14
2	MM_2	56.6±0.98	54±1.32	67.3±1.43
3	MM_3	55.1±1.17	58±1.51	69.8±1.72
4	MM_4	53.9±1.27	60±1.12	74.4±1.58
5	MM_5	52.6±1.20	63±1.06	79.6±1.29

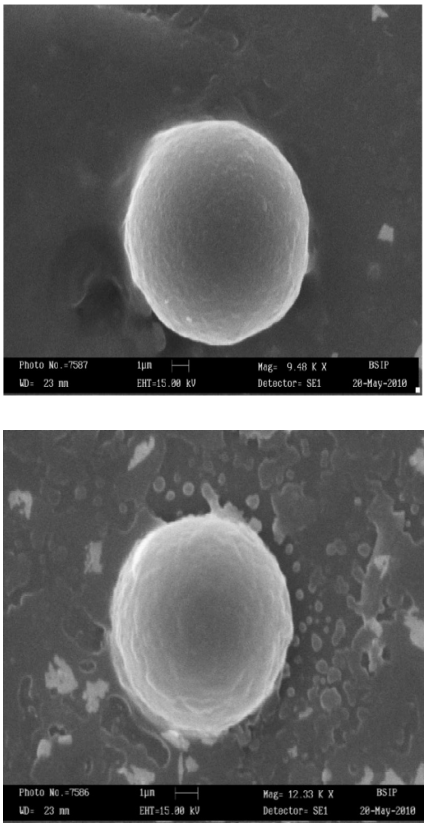

Fig. I: SEM photograph of Microspheres

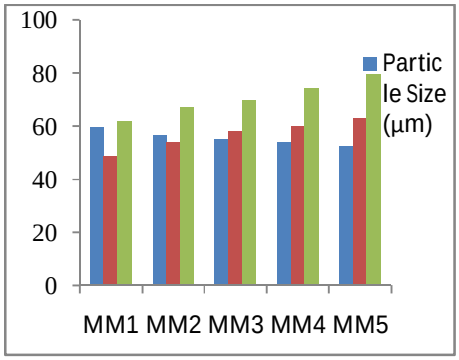

Fig. II: Comparative graph showing Particle size, Percentage drug entrapment and Percentage mucoadhesion of different formulations

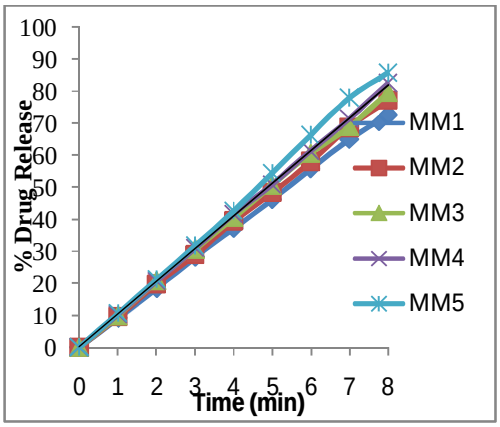

Fig. III: *In-vitro* drug release profile of different formulations showing the effect of drug and polymer on drug release of microspheres

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