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Formulation And Characterization of Clarithromycin Loaded Mucoadhesive Microspheres by Emulsification Solvent Evaporation for Anti-Helicobactor Pylori Therapy

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Helicobacter pylori (H. pylori) infection has a strong association with chronic active gastritis and duodenal ulcer (DU). H. pylori infection can be detected in 90% of patients with DU and 70% of those with gastric ulcers. The most successful and universal treatment is triple therapy, three d rugs twice a day for 1 week (proton pump inhibitor [PPI], amoxicillin and clarithromycin). However, some other reports and clinical trials indicate that the therapies cannot bring out complete eradication of H. pylori and suggest that the therapeutic effect needs more investigation. One of the reason for incomplete eradication is probably that the residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where H. pylori exists. One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach. Considering above issue, we have prepared clarithromycin loaded microspheres for anti – H.pylori Therapy. The microspheres were prepared by the w/o emulsification solvent evaporation method using mucoadhesive polymers sod. CMC and a release controlling polymer sod.alginate. The shape and surface morphology of prepared microspheres were characterized by optical microscopy . In vitro drug release studies were performed and drug release evaluated.

Keyword: Mucoadhesive Microspheres, Clarithromycin, In Vitro Release, Gastric Residence Time

INTRODUCTION: Microspheres are one of the particulate delivery systems used to achieve sustained or controlled drug delivery, improve bioavailability , stability and target drug to specific sites. Microspheres also offer advantages such as limiting fluctuation within a therapeutic range, reduction in side effects, decreased dose frequency and hence improved patient compliance[1,2]. The popular method for the encapsulation of drugs within water-insoluble

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polymers is the emulsion solvent evaporation method. This technique offers several advantages and is preferable to other preparation methods such as spray drying, sonication and homogenization because it requires only mild conditions such as ambient temperature and constant stirring. Thus, a stable emulsion can be formed without compromising the activity of the drugs.

Delivery of drugs directly to the colon is one of the areas of interest to present day researchers in pharmaceutical sciences. The colon targeted delivery of pharmaceutical drugs is important to achieve localized effect for the treatment of colonic diseases and/or systemic drug delivery for drugs absorbed from colon, for e.g. peptides and proteins. Proteins and peptides such as insulin, calcitonin and vasopressin may be delivered systematically via colonic absorption.
The therapeutic advantages of targeting the drug to the diseased organ include:
• Delivery of the drug in its intact form as close as possible to the target site.
• Reduces conventional dose and frequency
• Reduced incidence of adverse side effects.[3]

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 [4]

Most of the conventional drug delivery system for treating various disorders and diseases such as inflammatory bowel disease, colon cancer and intestinal amoebiosis have failed as drugs in its intact form as they do not reach the site of action in appropriate concentration. Microspheres drug administration offers a number of advantages in therapeutics, where the controlled releases of drug delivery as well as the predictable and reproducible drug release kinetics are important features of them in colon drug delivery system.

Clarithromycin is a semi-synthetic macrolide antibiotic. Chemically, it is 6-0 methylerythromycin.Its molecular formula is $C_{38}H_{69}NO_{13}$. Clarithromycin exerts its antibacterial action by binding to the 50S ribosomal subunit of susceptible microorganisms resulting in inhibition of protein synthesis.The dosage of clarithromycin is 500mg[5].

The purpose of this study was to design mucoadhesive microspheres containing clarithromycin as an anti-*H. pylori* agent and to evaluate the effectiveness of the mucoadhesive microspheres for *H. pylori* eradication therapy.

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000 µm in diameter and consist either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in stomach, thus offering the possibilities of localized as well as systemic controlled release of drugs. The application of mucoadhesive microspheres to the mucosal tissues of gastric epithelium is used for administration of drugs for localized action. Mucoadhesive microspheres are widely used because they release the drug for prolong period, reduce frequency of drug administration and improve the patient compliance[6].

MATERIAL AND METHODS:

Clarithromycin was gift sample from Om biotech (P Ltd.)Sidcul. Uttrakhand. sod.carboxymethyl cellulose (sod. CMC), sod. alginate, liquid paraffin, isopropyl alcohol, sodium hydroxide, acetone and dichloromethane was purchased from

Central Drug House(New Delhi) all the chemical were of analytical grade and double distilled water used throughout the experiment.

METHOD OF PREPARATION:

Formulation of microspheres with and without drug:

Microspheres were prepared by water in oil emulsification solvent evaporation technique. A 3% polymeric aqueous solution was made without drug and then the solution poured into 100 ml of light liquid paraffin containing 0.5% span-20 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker. Constant stirring at 500-1000 rpm was carried out using magnetic stirrer. The beaker and its content were heated at 500C, stirring and heating were maintained for 4.5 hrs. The aqueous phase was evaporated. The microspheres were washed with n-hexane, separated and dried at room temperature. The same procedure was done with drug[7].

S. No	Formulation	Drug	Sod. CMC	Sod. alginate
1.	A1	1	0.5	1.5
2.	A2	1	0.75	1.25
3.	A3	1	1.0	1
4.	A4	1	1.25	0.75
5.	**A5**	**1**	**1.5**	**0.5**

CHARACTERIZATION OF FORMULATION:

1. Morphology and Particle size Determination:

Particle size: Determination of average particle size of the clarithromycin microspheres was carried out by the optical microscope fitted with an ocular micrometer and stage micrometer.

2. Drug entrapment efficiency or Incorporation efficiency

The amount of clarithromycin present in the microspheres was determined by extracting the drug into phosphate buffer of pH 7.4 under magnetic stirring for a period of 2 h. The solution was filtered through Whatman filter paper no.5, suitably diluted. Clarithromycin content estimated by high performance liquid chromatography and the conditions for the HPLC assay were the same as before. The incorporation efficiency was calculated by the following formula:

Incorporation efficiency (%) = Experimental drug content × 100 /Theoretical drug content

3. Mucoadhesion study:

The in vitro mucoadhesive test was carried out using small intestine from chicken. The small intestinal tissue was excised and flushed with saline. Five centimeter segment of jejunum were everted using a glass rod. Ligature was placed at both ends of the segment. 100 microspheres were scattered uniformly on the everted sac from the position of 2 cm above. Then the sac was suspended in a 10ml tube containing 8 ml of saline by the wire, to immerse in the saline completely. The sac were incubated at 37^0C and agitated horizontally. The sac were taken out of the medium after immersion for 0.5, 1, 1.5, 2, and 2.5 hrs, immediately repositioned as before in a similar tube containing 8ml of fresh saline and unbound microspheres were counted. The adhering percent was presented by the following equation[8].

Mucoadhesion= (no. of microspheres adhered/no. of microspheres applied) ×100

4. In vitro drug release studies

Release of clarithromycin from the microspheres was studied in 0.1N HCL (900 mL) using a USP XXIII paddle method Dissolution Rate Test Apparatus with a rotating paddle stirrer at 50 rpm and 37° ± 1°C. A sample of microspheres equivalent to 25 mg of clarithromycin was used in each test. Samples of dissolution fluid were withdrawn through a filter (0.45 µm) at different time intervals and were assayed for drug release by high performance Liquid chromatography.

Table 2: Physicochemical characteristics of the Clarithromycin loaded mucoadhesive microspheres

Formulation code	Incorporation efficiency (%)± SD	Particle size (%)± SD	Mucoadhesion (mean ± SD) µm
A1	**68±1.56**	**612±1.38**	**64± 2.10**
A2	**83±2.31**	**655±2.12**	**67±2.37**
A3	**86±1.92**	**770±2.27**	**73± 2.43**
A4	**84±2.29**	**728± 3.15**	**80±1.13**
A5	**85±2.48**	**746±2.42**	**82±1.30**

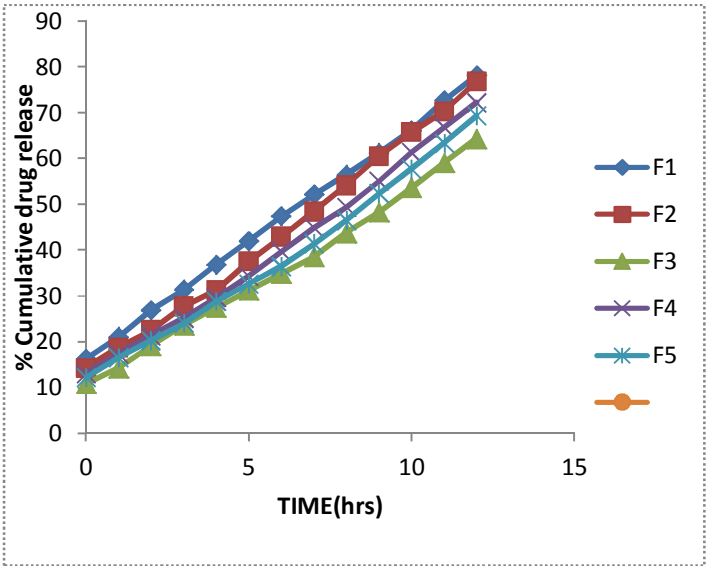
5. Angle of repose: Determination of angle of repose of clarithromycin microspheres were carried out by employing fixed funnel method.
Angle of repose θ = tan-1 (H/R)
H = Height of the pile
R = Radius of the pile

RESULT AND DISCUSSION:

The mucoadhesive microspheres of sod. alginate and sod. CMC prepared by solvent emulsification method. The polymer sod. alginate was selected to control the release rate and sod. CMC as a mucoadhesive polymer both are biodegradable and mucoadhesive polymer. The formulation of the present microspheres was based on the solubility behavior of both polymers. Five Formulation F1, F2, F3, F4 and F5 were formulated by varying concentration of sod. alginate and sod. CMC (as given in table no.1.), to study effect of polymer concentration on the size, percentage mucoadhesion, drug entrapment efficiency.

The resulting microspheres were found to be discrete, spherical, free flowing and of the monolithic matrix type. The microspheres were uniform in size with a mean size range of 612±1.38 to 746± 2.42 µm.

To assess the mucoadhesivity of the microspheres in-vitro wash off test was performed for all the formulations for 405 min (4hrs 15 min). Formulation F5 showed the highest mucoadhesivity 82% due to the presence of higher proportion of sod. CMC polymer, due to the anionic nature of the polymer, and F1 showed the lowest mucoadhesivity 64% due to higher proportion of sod. alginate due to the irregular surface was increased. The percent mucoadhesivity was found 64, 67, 73,80, 82% for formulation F1, F2, F3, F4 and F5 respectively, shown in table.



CONCLUSION:

H. pylori colonize the gastric mucosa leading to gastritis, gastric ulcer, and gastric carcinoma. To increase the efficacy of eradicating the infection, a localized delivery system of anti-*H. pylori* agents in the stomach is required. Clarithromycin microsphere formulation was prepared to increase the local concentration of the antibiotic in the stomach and, thus eradicate *H. pylori* infection. *In vitro* studies clearly indicates that the prepared formulations possess good bioadhesive properties. These properties enable the

microspheres to adhere to the gastric mucosal surface and stay in stomach for prolonged periods and could ensure the stability of drug in gastric environment, which eventually resulted in better eradication of *H. pylori* than the conventional dosage forms. Further studies are planned to examine the gastric residence time of the microsphere formulation and the efficacy in eradicating *H. pylori* infection in suitable animal model.

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