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Formulation and Evaluation of Clarithromycin loaded Mucoadhesive Microspheres for Anti- *Helicobacter pylori* Effect

Venkateswaramurthy N*, Sambathkumar R, Perumal P

JKK. Nataraja College of Pharmacy. Komarapalayam. Namakkal District -638183.Tamilnadu, India.

ABSTRACT

It is well documented that Helicobacter pylori infection can use chronic peptic ulceration and can increase the risk of gastric denocarcinoma. The wide use of antibiotic therapies for H. pylori infection has also increased the number of therapeutic failures. Recent data show a decreasing efficacy of these therapies worldwide. One reason for the incomplete eradication of *H. pylori* is probably due to the short residence time of antimicrobial agents in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists. The purpose of this study was to design mucoadhesive microspheres containing Clarithromycin as an anti-*H. pylori* agent to deliver the drug specifically to mucus layer where *H.pylori* resides and evaluate the effectiveness of the mucoadhesive microspheres for *H. pylori* eradication therapy. Microspheres were prepared by using Eudragit RL100 as matrix and Carbopol 974P and Hydroxy propyl methyl cellulose K4M as mucoadhesive polymer. The microspheres were prepared by emulsion solvent evaporation technique. The prepared microspheres were evaluated with respect to the particle size, production yield, encapsulation efficiency, shape and surface properties, mucoadhesive property, *In-vitro* drug release and suitability for anti *Helicobactor pylori* effect. The preliminary results show great promise for this delivery strategy in the treatment of *H. Pylori* infection.

Key words: clarithromycin, H.pylori, microsperes, mucoadhesive

*Corresponding author

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INTRODUCTION

Helicobacter pylori (H. pylori) is a Gram-negative bacterium that causes one of the most common chronic infections in humans. The infection produces chronic gastritis and predisposes to the development of peptic ulcer disease [1,2][1]. Clarithromycin has highest rate of eradication of H. pylori in monotherapy in vivo [3], 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 [4,5]. One reason for the incomplete eradication of *H. pylori* is probably due to the short residence time of antimicrobial agents in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists [6,7]. Mucoadhesive microspheres highly suitable drug delivery system for *H.pylori* eradication because it specifically bind with mucus where *H.pylori* resides and deliver the antibiotic for longer period. 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.

MATERIALS AND METHODS

Materials

Clarithromycin was gigted by Ranbaxy laboratories Ltd, New Delhi, India, Hydroxypropyl methyl cellulose K4M was obtained as gifts from Colorcon Asia Pvt. Ltd., Mumbai, India and Carbopol 974P was a gift from BF Goodrich Co., Germany. Eudragit RL 100 was a gift sample from Microlabs, Bangalore. All other reagents and chemicals used were of analytical grade.

Preparation of Microspheres

Microspheres were prepared by a solvent evaporation method. The solvent system acetone/liquid paraffin was used. Agglomeration of microspheres was prevented by using 1% w/v Span80. Eudragit RL 100 was used to form a matrix of microspheres and mucoadhesive polymer were chosen to produce mucoadhesion is Carbopol 974P and Hydroxypropyl methyl cellulose K4M. Eudragit RL 100 was dissolved in acetone and weighed quantity of Clarithromycin, Carbopol 974P and Hydroxypropyl methyl cellulose K4M were dispersed it. The total volume of acetone was 12 ml. This homogeneous final dispersion was cooled to 5 °C and poured slowly with stirring (700 rpm) into 80 ml of liquid paraffin containing 1% w/v span 80, which was previously also cooled to 5 °C. The obtained emulsion was stirred at 40 °C for 40 min. The suspension of microspheres in liquid paraffin was filtered and microspheres were washed by n-hexane and dried in vacuum at room temperature overnight.

Scanning electron microscopy

Scanning electron photomicrograph of Clarithromycin loaded mucoadhesive microspheres were taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification.

Particle size measurement

The prepared microspheres were sized by using a Malvern 2600 Laser Diffraction Spectrometer. The size of the microspheres was determined in n-hexane as a non-dissolving dispersion medium and the particles were suspended mechanically by magnetic stirring during the measurement.

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Degradation of clarithromycin in pH 1.2 [8]

The degradation rate of the antimicrobial agent at pH 1.2 was examined by reported method with slight modification. A known amount of clarithromycin was added to the medium, which was preheated at 37ºC±0.2°C, to make a final concentration of 10.0 µg/ml. An aliquot of the medium was withdrawn at predetermined time intervals and neutralized with a NaOH solution before being quantified by HPLC. Then the solution was filtrated through a 0.45 µm syringe filter then analyzed for clarithromycin content by reversed-phase high performance liquid chromatography (RPHPLC) method using a mobile phase consisting of acetonitrile–aqueous 0.05 M phosphate buffer solution of pH 4.0 (40:60 v/v). The apparatus used for HPLC analysis was an Agilent 1100 quaternary pump, with a variable wavelength detector, thermostatted autosampler and column thermostat. A Hypersil ODS C18 column (250mm×4.6mm ID, 5 µm, Thermo, UK) was fitted with a Phenomenex guard column packed with octadecyl C18 (Phenomenex, USA).The column temperature was maintained at 40°C and flow rate of 1ml/min. The The concentrations of the parent drug remaining were analyzed by RP-HPLC assay. The degradation of clarithromycin was assumed to follow pseudo-first order kinetics, which is described by the following equation:

$$C=C_o e^{-kt}$$

In which C is the concentration of clarithromycin remaining at time t, C_o is the initial concentration of clarithromycin, and k is the pseudo-first order degradation rate constant. The half-life ($t_{1/2}$) of clarithromycin was determined from the pseudo-first order degradation rate constant. Degradation rate constant used to correct the drug release data obtained in acidic media.

Determination of drug encapsulation efficiency

To determine the total drug content of microspheres a known amount of microspheres wereground to fine powder. Accurately weighed (50mg) grounded powder of microspheres weresoaked in 50 ml of distilled water and sonicated using probe sonicator for 2 h. The whole solution was centrifuged using a tabletop centrifuge to remove the polymeric debris. Then the polymeric debris was washed twice with fresh solvent (water) to extract any adhered drug. The clear supernatant solution was filtrated through a 0.45 µm syringe filter then analyzed for Clarithromycin content by high performance liquid chromatography and the conditions for the HPLC assay were the same as before.

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 (Dissco 2000, Labindia) 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.

In- vitro evaluation of mucoadhesivenes [9]

A strip of goat intestinal mucosa was mounted on a glass slide and accurately weighed mucoadhesive microspheres in dispersion form was placed on the mucosa of the intestine. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle 45^0. Phosphate buffer saline (pH 6.4), previously warmed to 37 ± 0.5^0C, was circulated to the cell over the microspheres and membrane at the rate of 1 ml/min with the help of pump. Washings were collected at different time intervals and microspheres were separated by centrifugation followed by drying at 50^0C. The weight of microspheres washed out was taken and percentage mucoadhesion was calculated by

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$$\text{Percentage mucoadhesion} = \frac{Wa - Wl \times 100}{Wa}$$

where Wa = weight of microspheres applied; W1 = weight of microspheres leached out.
iquid chromatography. The drug release experiments were conducted in triplicate (n = 3).

Table 1: Formulation composition of mucoadhesive microspheres of Clarithromycin

Formulation Code	Eudragit RL 100(%w/v)	Carbopol 974P(%w/v)	HPMC K4M* (%w/v)
F1	3	1.0	1.0
F2	5	1.0	1.0
F3	7	1.0	1.0
F4	5	0.5	0.5
F5	5	0.75	0.75
F6	5	1.5	1.5

HPMC = Hydroxypropyl methyl cellulose

Table. 2. Physico-chemical characteristics of the Clarithromycin loaded mucoadhesive microspheres

S.No	Formulation code	Mean Particle size (µm)	Drug Entrapment (%) ±S.D (n=3)	Mucoadhesion (%) ±S.D* (n=3)
1	F1	173	82± 1.13	83±2.178
2	F2	225	88±1.77	86±1.856
3	F3	337	90±2.11	87±1.775
4	F4	203	90±1.82	82±1.145
5	F5	274	86±1.61	91±0.987
6	F6	306	82±2.21	93±1.421

S.D = Standard deviation

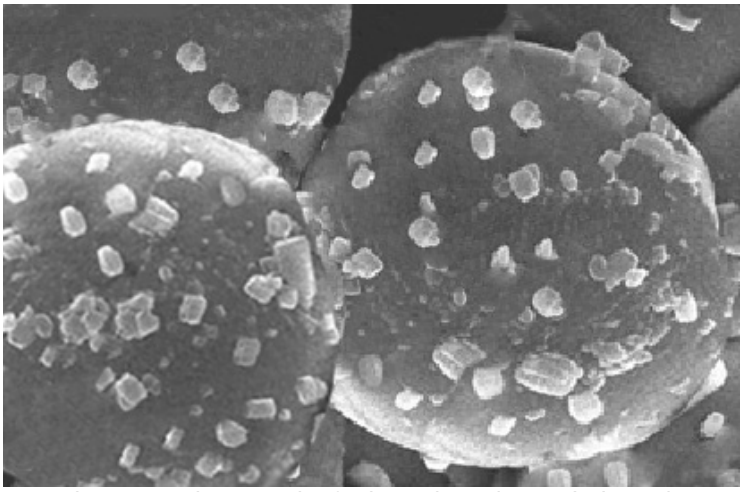

Fig 1. SEM photograph of microsphere (Formulation F6)

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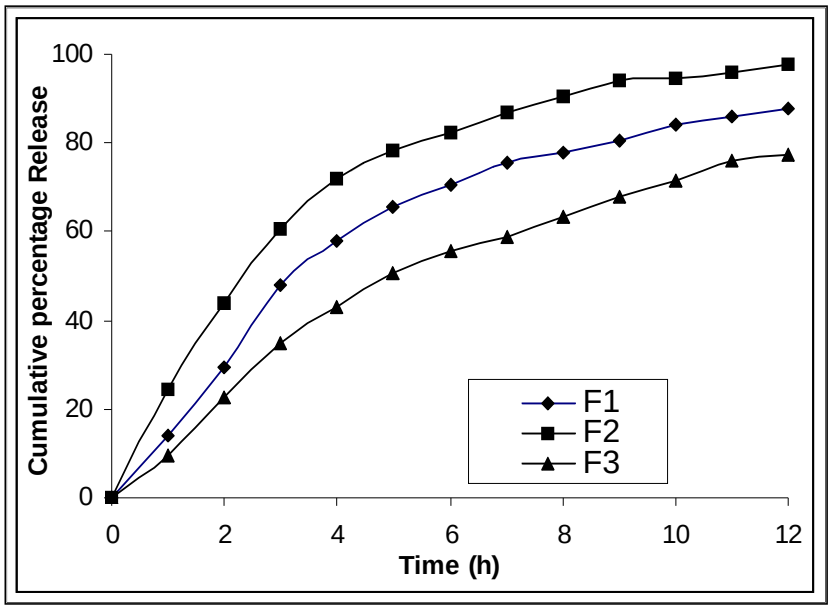

Fig.2 Effect of EudragitRL100 on the inv release of Clarithromycin in 0.1 N Hcl

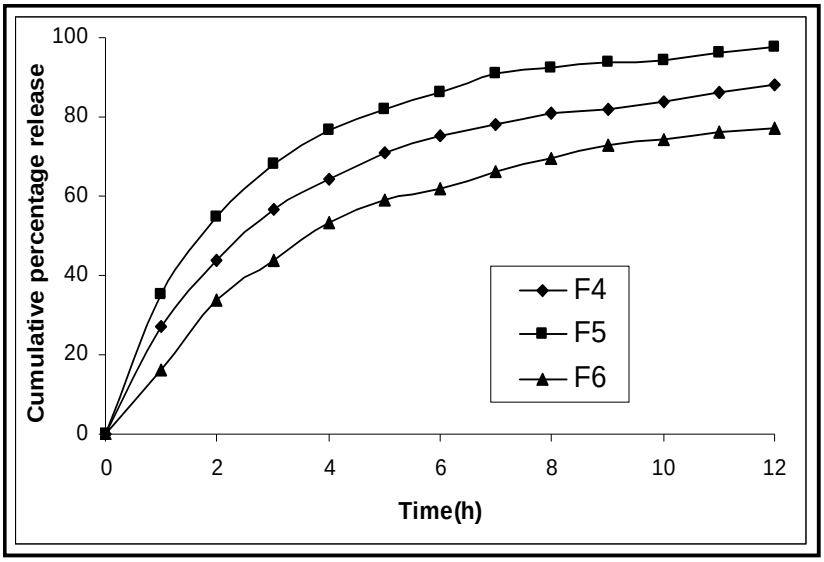

Fig.3 Effect of Mucoadhesive polymers on on the *In-vitro* release of Clarithromycin in 0.1 N HCL

RESULTS AND DISCUSSION

The mucoadhesive microspheres Clarithromycin prepared in this study were well-rounded spheres with the size ranging approximately from 17 to 337 µm. The study of In-vitro bioadhesion revealed that all the batches of prepared microspheres had good bioadhesive property ranging from 82±1.145 % to 93±1.421%. From the result of the *In-vitro* release test, the effect of Eudragit RL100 concentration on Clarithromycin release from were observed a significant decrease in the rate and extent of drug release was observed with the increase in polymer concentration in microspheres and could be attributed to increase in the density of the polymer matrix and also increase in the diffusional path length which the drug molecules have to traverse. Similarly, the effect of mucoadhesive polymers concentration on release properties of Clarithromycin were also studied. An increase in

mucoadhesive polymers concentration caused retardation in drug release from the microspheres because of an increase in the viscosity of polymer solution and formation larger size microspheres. The release of Clarithromycin from these batches were characterized by an initial phase of high release (burst effect) followed by a second phase of moderate release. *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 Clarithromycin in gastric environment, which eventually resulted in better eradication of *H. pylori* than the conventional dosage forms.

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