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Research Article

DESIGN, DEVELOPMENT AND EVALUATION OF AMOXICILLIN TRIHYDRATE MUCOADHESIVE MICROSPHERES FOR *HELICOBACTOR PYLORI* ERADICATION THERAPY

N.VENKATESWARAMURTHY*, R. SAMBATHKUMAR AND P. PERUMAL

Department of Pharmaceutics, J.K.K.Nataraja College of Pharmacy, Komarapalayam-638183, India. Email : murthyvenki@rediffmail.com

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ABSTRACT

In an effort to augment the anti *Helicobactor pylori* effect of Amoxicillin, mucoadhesive microspheres, which have the ability to reside in the gastrointestinal tract for an extended period, were prepared. Amoxicillin microspheres were prepared by using Eudragit RL100 as matrix and carbopol 974P 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, *invitro* drug release and suitability for anti *Helicobactor pylori* effect. In conclusion, mucoadhesive microspheres of amoxicillin prepared in this study could stay in the gastrointestinal tract for a longer period of time. Which indicate a potential use of mucoadhesive microspheres of amoxicillin in treating *H. pylori* infection.

Key words*: Helicobacter pylori*, Amoxicillin, mucoadhesive, microspheres

INTRODUCTION

Since the discovery of *Helicobacter pylori(H. pylori)* by Marshall and Warren[1], *H. pylori* is believed as a main microorganism causing gastric or peptic ulcers. Therefore, eradicating *H. pylori* is a prerequisite for curing a gastric or peptic ulcer and preventing a recurrence[2]

Amoxicillin was a semisynthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent. 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 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[3,4]. Therefore, some researchers had prepared and reported new amoxicillin formulations, such as float tablet, mucoadhesive tablet, pH-sensitive excipients composition mucoadhesive microspheres, etc., which were able to reside in the gastrointestinal tract for an extended period of time for a more effective *H. pylori* eradication. Among these formulations, mucoadhesive microspheres have gained considerable attention due to their ability to adhere to the mucus layer, as well as to release the drug in a sustained manner.[5]

The purpose of this study was to design amoxicillin mucoadhesive microspheres for *H. pylori* eradication therapy, to study the *in vitro* behavior of the microspheres, including, amoxicillin release, mucoadhesiveness.

MATERIALS AND METHODS

Materials

Amoxicillin Trihydrate was purchased from Zoetic formulations Ltd. Chennai, 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 adding 0.75% of Span80 . Eudragit RL 100 was used to form a matrix of microspheres and mucoadhesive polymer were chosen to produce mucoadhesion is carbopol 934P, Eudragit RS 100 was dissolved in acetone and mucoadhesive polymer was added as a powder. Amoxicillin Trihydrate in acetone were prepared separately and added to the dispersion of polymers. The total volume of acetone was 10 ml. The homogeneous final dispersion was cooled to 5 °C and poured slowly with stirring (900 rpm) into 80 ml of liquid paraffin, 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, microspheres were washed by *n*-hexane and dried in vacuum at room temperature overnight.

Amoxicillin stability in 0.1N HCl medium

It has been reported[6] that amoxicillin unstable in 0.1N HCl. Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from microspheres. Hence, in order to calculate correct amount of the drug release the degradation rate constant to be determined by following method. Weighed amount of amoxicillin (powder) was dissolved in 100 ml of 0.1N HCL and vibrated in a water bath maintained at 37^0 C. Then, HPLC assay was carried out 0, 1, 2 and 6 h, respectively, after amoxicillin was dissolved. The main peak area of amoxicillin at 1, 2 and 6 h was measured, respectively, and compared with that at 0 h. The degradation percentage of amoxicillin at different times in the 0.1N HCl medium and degradation rate constant were calculated. By using degradation rate constant actual drug release in 0.1N HCl were calculated.

The conditions for HPLC assay were as follow[7]: HPLC apparatus; P4000 (pump), UV6000LP (UV detector), ThermoFinnigan; column: Pinnacle II C18 5 µm 200×4.6 mm; mobile phase: phosphate buffer (0.01 M, pH 6.0): acetonitrile (96:4); flow rate: 1 mL/min. .The main peak area of Amoxicillin trihydrate at 1, 2, 4, and 8 h was measured and compared with that at 0 h.

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 amoxicillin content by high performance liquid chromatography and the conditions for the HPLC assay were the same as before.

Encapsulation efficiency was calculated using the following formula;

$$\text{Encapsulation efficiency} = \left(\frac{\text{Estimated drug content \%}}{\text{Theoretical drug content \%}} \text{x} 100 \right).$$

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.

***In vitro* evaluation of mucoadhesiveness[8]**

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

$$Percentage\ mucoadhesion = Wa\text{-}Wl\ X\ 100 \Big/ Wa$$

where Wa = weight of microspheres applied; W1 = weight of microspheres leached out.

Morphological characterization of microspheres

The surface view of the microspheres were observed via scanning electron microscopy(fig.1).

***In vitro* drug release studies**

Release of Amoxicillin 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 amoxicillin 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. The drug release experiments were conducted in triplicate (n = 3).

RESULTS AND DISCUSSION

The mucoadhesive microspheres of amoxicillin prepared in this study were well-rounded spheres with the size ranging approximately from 148 to 276 micrometer (Fig. 1).The study of *in vitro* bioadhesion revealed that all the batches of prepared microspheres had good bioadhesive property ranging from73±1.124 % to 92±0.221%. On increasing the mucoadhesive polymer concentration, the bioadhesive property of the microspheres also increased. The formulation F6 showed the highest bioadhesive property (92±.221%). These studies suggest that the spherical matrix of microspheres can interact with mucosubstrate on the surface of the stomach, and adhere to mucosa more strongly and could stay in stomach for prolong period for more effective *H. pylori* clearance.

Table 1: Formulation composition of mucoadhesive microspheres of Amoxicillin

Formulation code	Eudragit RL 100 (%w/v)	Carbopol 974P(%w/v)	Amoxicillin Trihydrate
F1	3	1.0	5
F2	5	1.0	5
F3	7	1.0	5
F4	5	0.5	5
F5	5	0.75	5
F6	5	1.5	5

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

S. No.	Formulation code	Mean particle size (µm)	Drug entrapment (%) ±S.D (n=3)	Mucoadhesion (%) ±S.D* (n=3)
1	F1	148	83± 2.23	81±1.178
2	F2	202	85±2.77	82±1.575
3	F3	317	87±3.11	85±1.886
4	F4	175	87±2.80	73±1.124
5	F5	250	85±1.63	78±0.785
6	F6	276	81±2.41	92±0.221

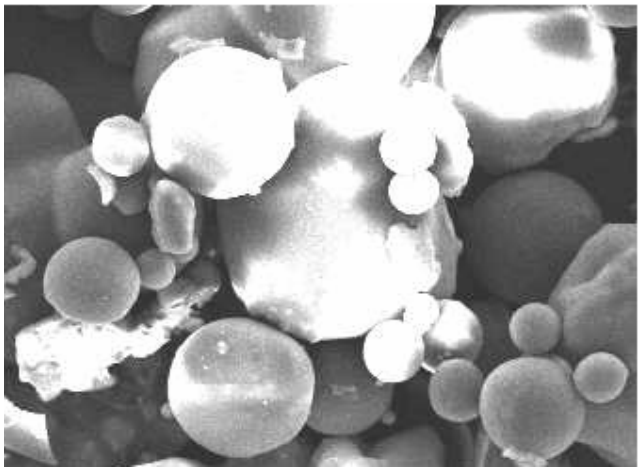

Fig. 1: Scanning Electron Micrograph (SEM) of the prepared Microspheres

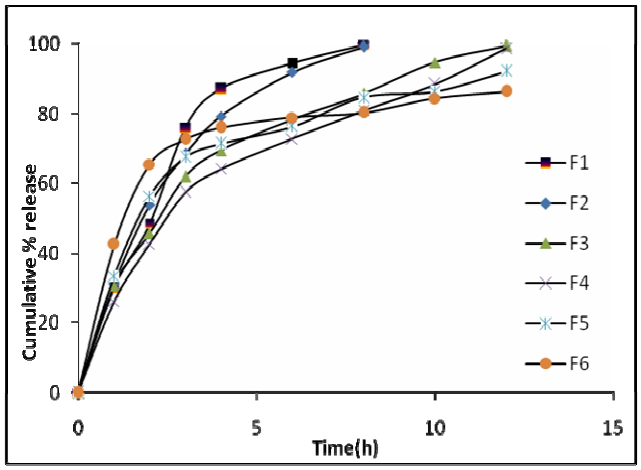

Fig. 2: Effect of EudragitRL100 and Carbopol 974P on the *invitro* release of Amoxicillin Trihydrate in 0.1 N HCL

It was reported that amoxicillin unstable in acidic (0.1N HCl) solutions[6]. Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from microspheres. Hence, in order to calculate correct amount of the drug release the degradation rate constant to be determined. The degradation profile of amoxicillin follows first order kinetics. The first order degradation constant and half-life of the degradation were calculated. The half life and degradation constant of amoxicillin trihydrate were found to be 7.127 hrs and 0.0972 hr^{-1}. These values used to correct the release profile of amoxicillin trihydrate. The following equation[9] was used to correct the amoxicillin release from microspheres in acidic solution (0.1N HCl).

$$dc/dt = dQ/Vdt - kC$$

Where C is the concentration of the drug at time t, Q the total amount of the drug released at time t, V the volume of the release medium, and k the first order degradation constant.

From the result of the *in vitro* release test, the effect of Eudragit RL100 concentration on Amoxicillin Trihydrate release from different batches of microspheres is shown in Fig. 2. 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 increase in the diffusional path length, which the drug molecules have to traverse. Similarly, an increase in mucoadhesive polymer concentration caused retardation in drug release from the microspheres because of an increase in the viscosity of polymer solution and formation larger size microspheres. In conclusion, mucoadhesive microspheres of amoxicillin prepared in this study could stay in the gastrointestinal tract for a longer period. Which indicate a potential use of mucoadhesive microspheres of amoxicillin in treating *H. pylori* infection.

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