



Design and Characterization of Amoxicillin trihydrate Mucoadhesive Microspheres for Prolonged Gastric retention

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ABSTRACT

Whilst there is keen interest in developing improved drug delivery systems to gastro intestinal tract for treatment of *Helicobacter pylori* induced peptic and duodenal ulcers, In an effort to augment the anti-*Helicobacter pylori* effect of Amoxicillin trihydrate mucoadhesive microspheres, which have the ability to reside in the gastrointestinal tract for an extended period, were prepared with ethyl cellulose as a matrix and carbopol 934P as a mucoadhesive polymer. Particle size was determined by optical micrometer and average particle size was found in the range of 500-560 µm for all batches. All the batches showed good in vitro mucoadhesive property. Cumulative percent drug release was found to be maximum for FI (91.12 %). Formulation FII found to follow Higuchi matrix with the regression value of 0.9985. From the all batches FI formulated microspheres showed more mucoadhesive property due to less amount of ethyl cellulose. In conclusion, the prolonged gastrointestinal residence time and controlled release might make contribution to *H. Pylori* clearance.

Keywords: Amoxicillin trihydrate, Carbopol 934P, *H. pylori*, Mucoadhesive microspheres.

INTRODUCTION

Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems. They have varied applications and are prepared using assorted polymers.^[1] However; the success of these microspheres is limited owing to their short residence time at the site of absorption. It would, therefore, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Bioadhesive microspheres have advantages such as efficient

absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site.^[2]

Helicobacter pylori are bacterium (gram) can infect the lining of the stomach and duodenum.^[3] Spiral-shaped, gram-negative bacterium *H. pylori* found in colonized gastric mucosa or adherent to the epithelial linings of the stomach.^[4] Gastric colonization by *H. pylori* is characterized by a lifelong extracellular persistence of the microorganism in a highly hostile, challenging ecological niche.^[5] *H. pylori* is a common infection, responsible for a variety of gastroduodenal pathway, duodenal and gastric ulcer, mucosa associated lymphoid tissue lymphoma, and gastric carcinoma. *H. pylori* infection produces an increase in basal and stimulated gastric acid output through a number of mechanisms, including gastrin, somatostatin, and inflammatory mediators. This phenomenon of increased acid output has been shown to occur in asymptomatic cases as well as those with peptic ulcer disease and nonulcer dyspepsia.^[6] Treatment of *H. pylori* is now effective but it can become resistant to common antibiotics and we need to develop strategies to stop this happening as well as finding

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alternative treatment for cases when resistance develops. Amoxicillin is a moderate-spectrum, bacteriolytic, β -lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β -lactam antibiotics. Amoxicillin is susceptible to degradation by β -lactamase-producing bacteria, about 20 % is bound to plasma proteins in the circulation and plasma half-life of 1 to 1.5 hours has been reported. [7-8]

Pursuing these objectives, this work was aimed at developing suitable slow release Amoxicillin trihydrate Mucoadhesive microspheres to reduce adverse effects, to enhance therapeutic efficacy and to avoid development of resistance.

MATERIALS AND METHODS

Amoxicillin trihydrate was purchased from LARK Laboratories (New Delhi, India); Ethyl cellulose was obtained from Colorcon Asia Pvt. Ltd (Goa., India). Carbopol 934P was obtained from Himedia Laboratories Pvt. Ltd (Mumbai, India). Span 80 and Heavy liquid paraffin were purchased from S.D Fine chemicals Ltd (Mumbai, India).

Methods

Preparation of Mucoadhesive microspheres

The Amoxicillin mucoadhesive microspheres were prepared by emulsification/evaporation method. [9] Accurately weighed quantity of ethyl cellulose (Table 1) was dissolved in 44.5 ml of acetone; 3 g of drug and 0.8 g of carbopol 934P powder were added to the ethyl cellulose solution with constant stirring for 24 hours. Then the suspension was slowly dispersed in 240 ml light paraffin containing 7.5 g, span 80 at a stirring rate of 600 rpm using propeller. After 30 minutes of emulsification, acetone was evaporated gradually with the help of a vacuum pump until the microspheres were formed. The system temperature was kept at 20°C throughout the process. The microspheres were washed with petroleum ether and dried at room temperature.

Table 1: Formulae for different batches of Amoxicillin trihydrate

Ingredients	Formulation code		
	FI	FII	FIII
Amoxicillin trihydrate	3 g	3 g	3 g
Ethyl cellulose	4.47 g	4.672 g	4.84 g
Carbopol 934P	0.8 g	0.8 g	0.8 g
Acetone	44.5 ml	44.5 ml	44.5 ml
Liquid paraffin heavy	240 ml	240 ml	240 ml
Span 80	7.5 g	7.5 g	7.5 g

Evaluation of prepared Mucoadhesive Amoxicillin microspheres

Particle size

All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope. [10]

The average particle size was determined using the Edmondsons equation.

$$D_{\text{mean}} = \sum nd / \sum n$$

Where n = No. of microspheres observed, d = mean size range

Determination of shape and surface morphology

The shape and surface morphology of the microspheres was studied by using scanning electron microscope. [11]

Drug content and Entrapment efficiency

Accurately weighed quantities of approximately 100 mg microspheres were dissolved in 100 mL phosphate buffer pH 7.4. The suspension were sonicated for 10 minutes, centrifuged at 4200 rpm for 30 minutes, and assayed at 272 nm. The encapsulated efficiency was calculated according to the following relationship. [12-13]

$$\text{Encapsulated efficiency} = \frac{\text{calculated drug concentration}}{\text{Theoretical drug content}} \times 100$$

In vitro evaluation of mucoadhesiveness of microspheres

The sheep stomach mucosa was used for *in vitro* mucoadhesion evaluation. [14-15] The mucosa was removed and cut into pieces 2 cm long and 1 cm wide and were rinsed with 2 ml of physiological saline. One hundred microspheres of each were scattered uniformly on the surface of the stomach mucosa. Then, the mucosa with the microspheres was placed in a chamber maintained at 93 % relative humidity and room temperature. After 20 minutes, the tissue were taken out and fixed on a polyethylene support at an angle 45°. The stomach was rinsed with pH 1.2 hydrochloric acid buffer for 5 minutes at a rate of 22 ml/ minutes. The microspheres adhered on to the surface of mucosa was counted, and the percentage of the adhered microspheres was calculated.

In vitro drug release studies

In vitro release studies were carried in pH 1.2 HCl buffer medium. Drug released rate was tested on all prepared formulations. The test conditions as follows: microspheres containing 100 mg of drug were placed in baskets in a vessel containing 900 ml of pH 1.2 HCl buffer medium with the temperature maintained at 37±0.5°C. The rotating rate of the basket was adjusted to 100 rpm. With intervals, 5 ml of samples were withdrawn and filtered through a whatman's filter paper. The equivalent volume of the medium with the same temperature was added to the dissolution vessel. The absorbance values of the filtrate at the wavelength of 272 nm were determined after suitable dilution. To analyze the mechanism and order of drug release from the microspheres, the data analysis was carried using PCOP dissolution software. [9]

RESULTS AND DISCUSSIONS

Particle size analysis

Hundred microspheres of each batch were sized and the average particle size was calculated. The mean size range of microspheres was found to be between 500-560 μm for all formulations (Table 2). Increase in the particle size was observed with increase in polymer concentration that might be due to more viscous nature of polymer solution.

Determination of shape and surface morphology

Scanning electron microscopy of prepared microspheres shows the spherical shape of microspheres with a slightly rough surface which may be because of surface associated drug crystals (Fig. 1A &1B).

Drug content and Entrapment efficiency

The entrapment efficiencies of all formulation are showed in Table 2. It was observed that increase in the concentration of the polymer increase the entrapment efficacy. This may be due to increase in the viscosity of the solution, which brought about the decrease in the emulsification leading to the formation of bigger globules in the emulsion.

In vitro mucoadhesion

Form the all batches FI formulated microspheres showed more mucoadhesive property due to less amount of ethyl cellulose (Table 2) and FII & FIII formulated microspheres

Table 2: Percentage yield (%), Average particle size (μm), Drug content (% w/w), Total entrapment efficiency (%), In vitro % mucoadhesion of prepared mucoadhesive amoxicillin microspheres

Formulation code	Percentage yield (%)	Average particle size (μm)	Drug content (% w/w)	Total entrapment efficiency	In vitro % mucoadhesion
FI	79.45	520.45 $\pm$ 1.21	28.4 $\pm$ 1.74	78.45 $\pm$ 1.6	91.85 $\pm$ 2.4
FII	78.13	521.28 $\pm$ 1.45	28.75 $\pm$ 1.25	79.41 $\pm$ 0.46	90.10 $\pm$ 3.2
FIII	78.95	551.25 $\pm$ 1.75	31.24 $\pm$ 0.54	86.29 $\pm$ 1.19	84.35 $\pm$ 2.1

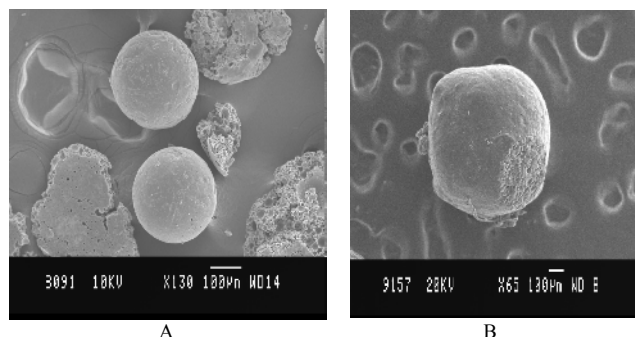
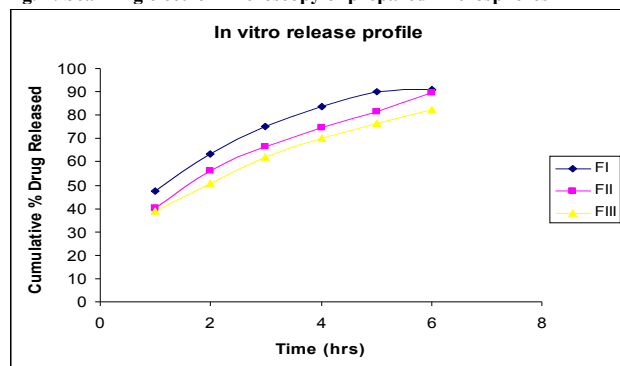
$\pm$ S.D- Standard deviation for (n=3)

Table 3: In vitro release profile (Cumulative % drug release) of formulations

Time (hrs)	Cumulative % Drug Release		
	FI	FII	FIII
1	47.35	40.3	38.95
2	63.45	56.05	50.55
3	75.25	66.54	61.8
4	83.5	74.8	70.35
5	89.9	81.25	76.65
6	91.12	89.45	82.15

Table 4: Model Fitting of Release Profile

Formulation code	Mathematical Models (r-values)			
	First order	Zero order	Higuchi matrix	Hixson-Crowell model
FI	0.9864	0.9875	0.9844	0.9853
FII	0.9832	0.9855	0.9985	0.9785
FIII	0.9994	0.9834	0.9974	0.9976

**Fig. 1: Scanning electron microscopy of prepared Microspheres****Fig. 2: In vitro release profile (Cumulative % drug release) of formulations**

showed less mucoadhesive property this may be because of decrease in the concentration of mucoadhesive polymer.

In vitro drug release studies

The release pattern of all the formulations were observed to be in a biphasic manner (Fig. 2) characterized by initial burst effect followed by a slow release. The burst effect corresponds to the release of the drug located on or near surface of the microspheres or release of poorly entrapped drug. The slow release period may be due to the drug diffusing out of the microspheres. The cumulative percent release after 6 hours was 91.12 %, 89.45 %, 82.15 % (Table

3) for FI, FII, FIII respectively this results are due to the increasing concentration of ethyl cellulose.

The regression co-efficient values of all formulations are shown in Table 4. These results indicate that FI followed zero order, FII followed Higuchi model, and FIII followed first order. All formulations shown release up to 6 hours.

From the experimental results it can conclude that formulation FI showed maximum percentage yield. Increase in the amount of polymer concentration added to the formulation increased entrapment efficacy for the drug. FIII showed maximum entrapment but decrease in mucoadhesive property. All formulations in suitable size range for administration. From the results of in vitro mucoadhesion and release profile we can conclude that these formulations can remain for longer period of time in stomach and release the drug in controlled manner.

From above studies it is concluded that stomach mucoadhesive drug delivery systems can be a suitable approach for the treatment of *Helicobacter pylori*.

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