

## Mucoadhesive Microparticulate Drug Delivery System of Esomeprazole Against Helicobacter Pylori Infection: Design, Development and Optimization

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### Abstract

The purpose of the present research was to develop and characterize mucoadhesive microspheres of esomeprazole for the potential use of treating gastric adenocarcinoma, gastric and duodenal ulcer associated with Helicobacter pylori. Esomeprazole mucoadhesive microspheres were prepared using HPMC, Chitosan and Acrycoat S 100 as a by an emulsion-solvent evaporation technique. The factorial designing methodology was used for optimization of formulation for two factors at three levels each was employed to study the effect of independent variables, polymer1: polymer2: emulsifier ratio (chitosan: acrycoatS100) (X1) and surfactant concentration: tween80 (X2) on dependent variables, namely particle size, drug entrapment efficiency, percentage mucoadhesion and in vitro drug release. Optimized formulation was obtained using desirability approach of numerical optimization. The drug release was also found to be slow and extended more than 8 h and release rates were fitted to the Power law equation and Higuchi model to compute the diffusional parameters. The prolonged stomach residence time of esomeprazole mucoadhesive microspheres might make a contribution to H. pylori complete eradication in combination with other antimicrobial agents.

**Key words:** Esomeprazole, Helicobacter pylori, mucoadhesive

### Introduction

Helicobacter pylori (H. pylori) is a gram-negative helical rod that colonizes the human gastric mucous layer. H. pylori is reported to be an important etiologic factor in the development of the chronic gastritis, ulcers (gastric and duodenal) and gastric adenocarcinoma [1]. H. pylori carriage rates are about 80-90% in developing countries with high risk of gastric cancer. Although the microorganism is susceptible to many antimicrobial agents but clinical trials with a single antimicrobial agent have resulted in a low eradication rate of H. pylori [2]. The most effective therapies of H. pylori infection require a minimum of two antibiotics in combination with a gastric acid inhibitor. Treatment with this therapy suffers from various side effects, namely nausea, drug resistance, infection recurrence, stomach upset and diarrhea. Degradation of antimicrobial (e.g. amoxicillin and clarithromycin by gastric acid) and short residence time of antimicrobial agents in the stomach are two major reasons for the failure of H. pylori eradication [3]. Resistance to antibiotics particularly to metronidazole and clarithromycin is also an increasing problem. Because of the undesirable side effects, resistance to antibiotics and high cost of the therapy, makes it very difficult for the effective management of the condition. Therefore, there is an enormous requisite to search for safe, non-toxic, inexpensive and effective non-antibiotic compounds that can inhibit growth of bacteria i.e. H. pylori. Esomeprazole exerts its stomach acid-suppressing effects by preventing the final step in gastric acid production by covalently binding to sulfhydryl groups of cysteines found on the (H<sup>+</sup>, K<sup>+</sup>)-ATPase enzyme at the secretory surface of gastric parietal cells [4]. This effect leads to inhibition of both basal and stimulated gastric acid secretion, irrespective of the stimulus. As the binding of esomeprazole to the (H<sup>+</sup>, K<sup>+</sup>)-ATPase enzyme is irreversible and new enzyme needs to be expressed in order to resume acid secretion, esomeprazole's duration of antisecretory effect persists longer than 24 hours. PPIs such as esomeprazole have also been shown to inhibit the activity of dimethylarginine dimethylaminohydrolase (DDAH), an enzyme necessary for cardiovascular health [5]. DDAH inhibition causes a consequent accumulation of the nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA), which is thought to cause the association of PPIs with increased risk of cardiovascular events in patients with unstable coronary syndromes [6]. Esomeprazole is a proton pump inhibitor used to treat GERD, reduce the risk of NSAID associated gastric ulcers, eradicate H. pylori, and to treat conditions causing gastric acid hypersecretion. Their study highlights the potential of esomeprazole-based therapy as a promising anti-H. pylori agent having property to restore and repair the gastric damage caused by H. pylori infection. Increasing

complications in the conventional antibiotic combination therapy stimulate an urgent need to develop new non-antibiotic antibacterial agents against *H. pylori* infection in order to provide safe, effective and specific cellular target moieties [7]. The multiparticulate can greatly improve the pharmacotherapy of the stomach through local drug release, leading to high drug concentration at the gastric mucosa, thus eradicating *H. pylori* from the sub-mucosal tissue of the stomach and making it possible to treat stomach and duodenal ulcer, gastritis and esophagitis. In the present study the bioadhesive system for esomeprazole was designed, optimized and evaluated.

## Materials and methods

### Materials

Esomeprazole was obtained from Intas Pharm. Pvt. Ltd, India. HPMC, chitosan: acrycoatS100 were obtained from Himedia, India. Double distilled water was used in all the preparations. All other solvents and chemicals used were of analytical grade.

### Methods

**Preparation of mucoadhesive esomeprazole microspheres:** Mucoadhesive microspheres of esomeprazole were prepared containing chitosan: acrycoatS100 by emulsion-solvent evaporation technique. Briefly, HPMC was dissolved in a water medium with amount of drug esomeprazole. The polymers chitosan was added to the above polymeric solution under stirring. AcrycoatS100 was dissolve in acetone and added into previous mixture. In batches EMM1 – EMM8 the drug-to-polymer (Chitosan: actycoatS100) ratio. The resultant mixture was extruded through a syringe (# 20) in a mixture of liquid paraffin (mixture of heavy and light, 1:1) containing 0.5 to 1.5% v/v of Span 60 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm for 3 hours. The microspheres formed were then dried at room temperature. Prepared microspheres were collected by filtration and washed several times with petroleum ether to remove liquid paraffin. The microspheres were then dried at room temperature (at 25°C and 60% relative humidity) for 24 hours [8].

**Experimental design:** A central composite design was employed for optimization of formulation. The chosen independent factors, i.e., drug:polymer:polymer ratio (Esomeprazole: chitosan: acrycoatS100) (X1) and tween 80 concentration (X2). Multiparticulate approaches include formulations in the form of pellets, granules, beads, microparticles and more significant effects on the property of microspheres.

### Characterization of microspheres:

**Fourier transform infrared spectroscopy (FTIR):** FTIR spectra of esomeprazole and EC/Carbopol 934P microspheres were recorded using FTIR spectrophotometer (Agilent Cary 630 FTIR Spectrometer) to investigate any interaction between esomeprazole and polymers in formulated microspheres. The scanning range was 4000 to 650 cm<sup>-1</sup> and the resolution was 8 cm<sup>-1</sup> [9].

**Estimation of esomeprazole:** Esomeprazole was estimated by ultraviolet visible (UV/Vis) spectrophotometric method (Systronic 2202) based on the measurement of absorbance at 262 nanometers. The method was validated for linearity, accuracy and precision. The method obeys Beer's law in the concentration range of 2-20 µg/ml [10].

**Drug entrapment efficiency:** Accurately weighed (50 mg) washed microspheres were crushed in a glass mortar pestle and the powdered microspheres were suspended in 10 ml of methanol and kept for 12 h. After filtration appropriate dilution were made with methanol and the absorbance was measured spectrophotometrically at 262 nm against blank [11-12]. The entrapment efficiency was calculated using the following formula:

$$\text{Entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

**Particle size of microspheres:** About 50 microspheres were spread (50) onto the wet, rinsed, tissue specimen and allowed to hydrate. The prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, which contained the gastric fluid (pH 1.2). At the end of 30 min, 1 h and at hourly intervals up to 8 h, the number of microspheres still adhering onto the tissue was counted [13].

**Drug release study:** The release of esomeprazole from the microspheres was investigated by using USP XXIV

basket apparatus (Electrolab, Mumbai, India) at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  and at 100 rpm using 900 mL of 0.1N HCl (pH 1.2) as a dissolution medium. Microspheres equivalent to 50 mg of esomeprazole were used for the test. Sample solution (5 ml) was withdrawn at pre-determined time intervals (every 30 min for 6 h and at hourly intervals up to 8 h), filtered through a  $0.45\ \mu\text{m}$  membrane filter, diluted and analyzed spectrophotometrically against the blank treated under similar conditions. An equal amount of fresh medium was replaced immediately after withdrawal of the test sample [14-15].

**Release kinetics:** Data obtained from in vitro release studies were fitted to various kinetic equations to find the mechanism of drug release from the microspheres. The kinetic models used were zero-order, first-order, Higuchi's model, Korsmeyer-Peppas model and Hixson-Crowell cube-root models. The release rate constants (k) and determination coefficients (r) were calculated by means of PCP disso v2.08 software. Curve fitting was performed using PCP disso v2.08 software [16].

## Result And Discussion

FTIR analysis revealed that there was no interaction between the drug and the polymers, thus these polymers can be conveniently used in further development of stomach specific mucoadhesive esomeprazole microspheres. The prominent spectrum of esomeprazole is 3875.56, 3789.89, 3455.11 NH stretch; 3147.51 O-H Stretch; 2937.22, 2980.62, 2902.11 CH stretch; 1709.02, 1746.59, 1698.13 C=O stretch; 1639.48, 1602.95 C=C stretch; 1544.65 S=O stretch; 1452.45, 1508.42 N-H out of plane; 1357.47, 1251.12 C-H bend; 1166.47, 1152.66 C-N stretch; 993.26, 912.63, 850.48 N-H bend, O-H bend; 1413.52 CH<sub>2</sub>,CH<sub>3</sub>. Mucoadhesive microspheres of esomeprazole were prepared by emulsion-solvent evaporation technique. Microspheres obtained were free flowing in nature. Preliminary trials were carried out to optimize the solvent ratio. Surfactants like tween 80 was found to be suitable surfactant for formulation of mucoadhesive. The mean size range of microspheres was found to be between  $87 \pm 2.47$  and  $141 \pm 3.14\ \mu\text{m}$  for all formulations. Increase in the particle size was observed with increase in polymer concentration that might be due to more viscous nature of polymer solution. The entrapment efficiencies of all formulation observed that increase in the concentration of the polymer increases 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.

**Table 1: Preparation of mucoadhesive microspheres**

| F. Code | Chitosan (mg) | Acrycoat S 100 (mg) | Tween 80 |
|---------|---------------|---------------------|----------|
| EMM1    | 500           | 0                   | 5        |
| EMM2    | 1000          | 0                   | 5        |
| EMM3    | 0             | 500                 | 5        |
| EMM4    | 0             | 1000                | 5        |
| EMM5    | 500           | 0                   | 10       |
| EMM6    | 1000          | 0                   | 10       |
| EMM7    | 0             | 500                 | 10       |
| EMM8    | 0             | 1000                | 10       |

**Table 2: Characterization of mucoadhesive microspheres**

| F. Code | Mean particle size <sup>b</sup> ( $\mu\text{m}$ ) | Bulk density <sup>a</sup> ( $\text{g}/\text{cm}^3$ ) | Tapped density <sup>a</sup> ( $\text{g}/\text{cm}^3$ ) | Carr's index <sup>a</sup> (%) | Angle of repose <sup>a</sup> ( $^{\circ}$ ) | Percentage yield | Percent entrapment | Degree of swelling |
|---------|---------------------------------------------------|------------------------------------------------------|--------------------------------------------------------|-------------------------------|---------------------------------------------|------------------|--------------------|--------------------|
| EMM 1   | $256.02 \pm 3.18$                                 | $0.38 \pm 0.014$                                     | $0.43 \pm 0.024$                                       | $11.62 \pm 1.15$              | $23.21 \pm 1.11^{\circ}$                    | $88.87 \pm 2.68$ | $85.04 \pm 1.36$   | $8.01 \pm 2.22$    |
| EMM 2   | $254.68 \pm 2.63$                                 | $0.45 \pm 0.015$                                     | $0.45 \pm 0.018$                                       | $17.28 \pm 0.15$              | $27.12 \pm 1.31^{\circ}$                    | $94.2 \pm 2.69$  | $88.9 \pm 2.01$    | $31.03 \pm 2.22$   |
| EMM 3   | $252.12 \pm 3.81$                                 | $0.43 \pm 0.011$                                     | $0.49 \pm 0.021$                                       | $12.24 \pm 0.18$              | $23.25 \pm 1.02^{\circ}$                    | $88.87 \pm 2.68$ | $85.27 \pm 1.41$   | $8.76 \pm 1.22$    |
| EMM 4   | $251.15 \pm 3.87$                                 | $0.49 \pm 0.011$                                     | $0.51 \pm 0.011$                                       | $12.00 \pm 0.38$              | $27.54 \pm 1.59^{\circ}$                    | $95.2 \pm 2.69$  | $90.14 \pm 1.14$   | $1.79 \pm 0.55$    |

|       |                 |                |                |                |                 |           |            |                |
|-------|-----------------|----------------|----------------|----------------|-----------------|-----------|------------|----------------|
| EMM 5 | 259.02±2.1<br>3 | 0.41±0.00<br>4 | 0.47±0.01<br>7 | 12.76±<br>0.39 | 23.18±1.08<br>° | 90.1±2.61 | 86.21±1.76 | 9.02 ±1.13     |
| EMM 6 | 261.18±2.0<br>3 | 0.39±0.01<br>7 | 0.44±0.02<br>2 | 13.33±1.4<br>7 | 27.11±1.80<br>° | 97.7±2.64 | 91.09±1.46 | 37.21<br>±2.22 |
| EMM 7 | 258.13±3.5<br>1 | 0.42±0.02<br>1 | 0.48±0.02<br>4 | 12.51±0.2<br>7 | 23.01±1.11<br>° | 80.1±1.03 | 81.85±2.06 | 3.11±1.01      |
| EMM 8 | 138.07±4.0<br>5 | 0.42±0.02<br>8 | 0.55±0.01<br>9 | 13.10±0.1<br>2 | 27.08±1.63<br>° | 78.5±1.01 | 86.18±.74  | 1.93 ±0.94     |

Figure 1: FTIR spectra of drug esomeprazole

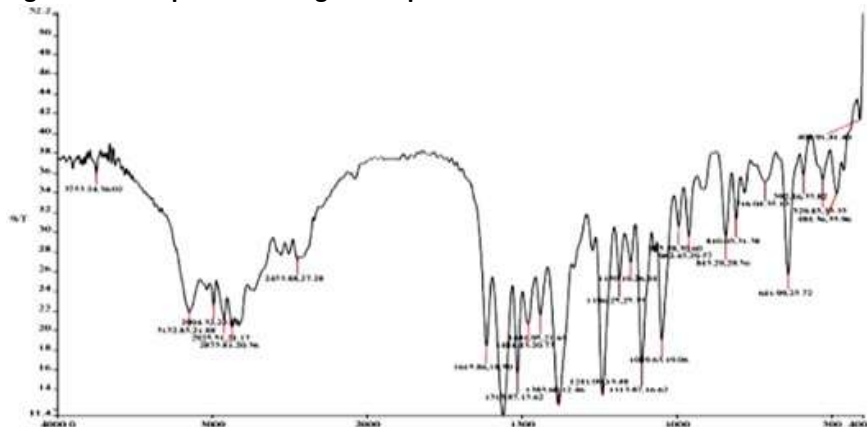


Figure 2: Standard Curve of drug in simulated gastric fluid (pH 1.2) at 262.0 nm

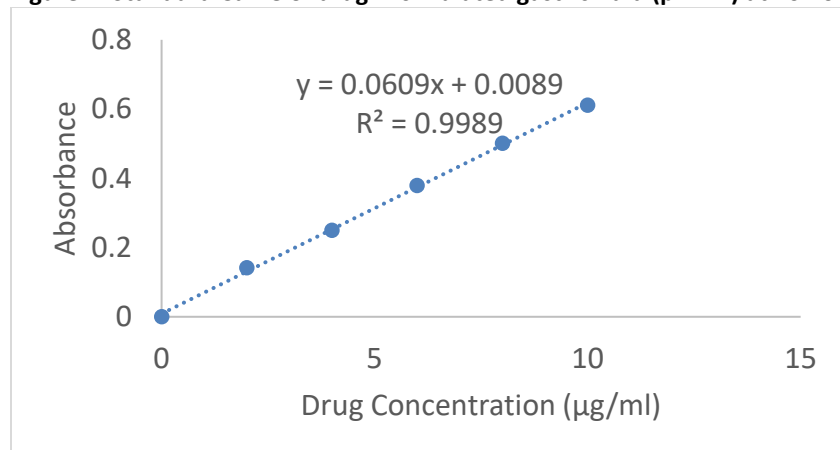
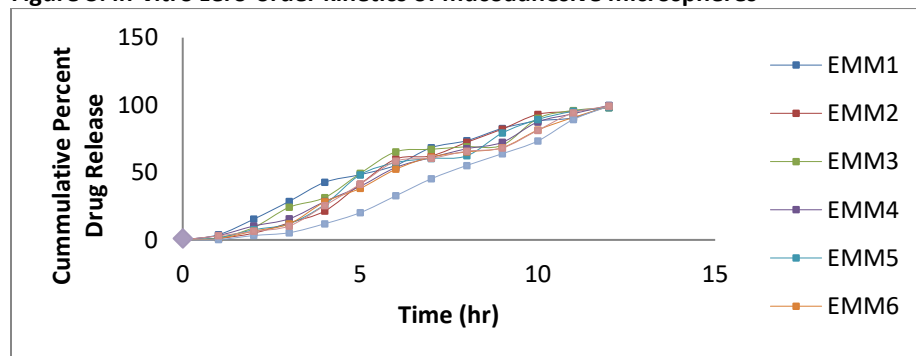


Figure 3: in-vitro zero-order kinetics of mucoadhesive microspheres



## Conclusion

The optimized formulation for esomeprazole mucoadhesive microspheres was obtained with Esomeprazole: chitosan: acrycoatS100 using response surface methodology based on a central composite design. It was found that the optimized formulation was achieved and the observed responses were close to the predicted values for the optimized formulation. The drug release from the optimized formulation showed a controlled-release pattern which could offer both local and systemic action for effective treatment of *H. pylori* infection. Increasing complications in the conventional triple-therapy (TT) stimulate an urgent need to develop new non-antibiotic antibacterial agents against *H. pylori* infection that are safe, highly effective.

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