Formulation and In Vitro evaluation of pH trigger polymeric blended buoyant beads of clarithromycin

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Abstract: Helicobacter pylori (H. pylori) infections are the leading cause of gastro-duodenal disease which is potentially life threatening, and spreading all over the world. The purpose of this study was to develop pH-sensitive floating-bioadhesive drug delivery system to increase the efficacy of clarithromycin against H. pylori. In the present investigation buoyant beads of pectin, wherein the oil was blended with hydroxypropyl methyl cellulose (HPMC) or carbopol 934. The micro gel beads were prepared by ionotropic gelation technique using calcium carbonate as gas forming agent and the drug polymer dispersion was emulsified with mineral oil. The study of scanning electron microscopy revealed that prepared beads were dense spherical with minor wrinkles on entire surface of the beads. The formulation prepared by using blend of polymers comprising pectin-carbopol was found to have larger diameter, increased % floating, higher encapsulation efficiency and drug content. In vitro clarithromycin release study of gel beads was carried out in simulated gastric fluid and in phthalate buffer of pH 3.4 for a period of 8 hr. When tested in Mongolian gerbils rats, ethyl cellulose coated optimize beads were found to have higher mucosal drug retention and significantly eradicated H. pylori from stomach of the treated animals. The results indicate that controlled release gastro-retentive optimized beads proved to be a possible candidate delivery system for effective eradication of H. pylori infection.

Keywords: Anti-infectious agent, Polymer blend dispersion, Stimuli trigger drug delivery system , In vivo evaluation, Gastric transit time, Bacterial cell line.

Introduction

Oral route of administration still remains the route of choice for the majority of clinical application and for the local action in the gastrointestinal tract. Indeed, for controlled release system, oral route of administration has received the more attention and success because gastrointestinal physiology offers more flexibility in dosage form design than other routes\textsuperscript{1}. Novel oral controlled dosage form that is retained in the stomach for prolonged and predictable period is of major interest among formulation scientist. One of the most feasible approaches for achieving prolonged and predictable drug delivery in the gastrointestinal (GI) is to control gastric residence time (GRT). Dosage form with prolonged GRT or gastroretentive dosage form provides an important therapeutic option\textsuperscript{2}. 
Figure 1. Schematics of targeted drug delivery approach for \textit{H. pylori} eradication located within the stomach.

\textit{H. pylori} is a microaerophilic, spiral and gram-negative bacillus with a 4-6 bulbous tipped sheathed flagella at one end, which helps it to penetrate the gastric mucosa and colonize on the gastric antrum. The bacterium adheres to the gastric epithelial cells and remains on the luminal surface of the gastric mucosa under the mucus gel layer, and access of antimicrobial drug to the site is restricted both from the stomach and from the gastric blood supply. Therefore, research scientists are consistently striving to search novel facet for targeted delivery of antimicrobial agent for the cure of \textit{H. pylori} infection in the stomach and duodenum (Figure 1).

The pH range of the gastric fluids varied in the different segment of the GI and this may provide environmental stimuli for targeted release of the drug from the delivery system. Oral pH trigger controlled drug delivery systems can maintain a higher drug concentration in the gastric region where \textit{H. pylori} exists and thereby improve the therapeutic efficacy. In the present study oil entrapped buoyant gel beads of clarithromycin were design, wherein hydrophilic polymer (carbopol 934P or HPMC) was blended with pectin in order to modify the delivery system as gastroretentive and floating. The prepared delivery system was expected to plug and seal the infected and inflamed mucosal cells by penetration of clarithromycin in side the infected cells and the drug was released in a sustained manner. The aim of the present work was to develop \textit{pH} sensitive gastroretentive microbeads of CI for controlled and site specific delivery of drug to treat colonization of \textit{H. pylori}. To achieve this objective an ethylcellulose (EC) coated optimize microbeads of CI was prepared in order to treat the colonization of \textit{H. pylori}.

**Material and Methods**

**Materials**

Clarithromycin was obtained as gift sample from Ranbaxy Laboratories Ltd, Gurgoan, India. Carbopol 934P and Hydroxypropyl methylcellulose K4M were obtained as a gift sample from Ranbaxy laboratory Devash, India. Low methoxy pectin, calcium chloride, and ethyl cellulose were obtained from S.D. Fine Chem India. Light mineral oil was obtained from the Central Drug House, India. Brucella broth and fetal calf serum were purchased from Himedia Mumbai, India. Agarose was purchased from FMC BioProducts Rockland, USA. All other ingredients, reagents and solvents were of analytical grade.

**Animals**

Seven weeks old specific pathogen free male Mongolian gerbils with body mass 65 ± 6 g were used for the present study. The animals were housed in four to five cages in air conditioned room in which the temperature (24 ± 2°C) and humidity (60 ± 5%) with free access to standard commercial rodent diet and tap water.
Methods

Preparation and optimization of microgel beads
The beads were prepared by ionic gelation method by successively mixing of aqueous pectin solution (1.5-2.0 w/v) into HPMC or carbopol 934P in order to prepare specific ratio of blended polymeric dispersion with continue stirrings up to 15 min (Table 1). The drug (0.70% w/v) and calcium carbonate (0.5-1.00% w/w) were dispersed into 20 ml of polymeric blended dispersion and was emulsified with 05-15% w/v of light mineral oil. The beads were collected and coated with ethyl cellulose by the published method with slight modification. The coating variables are presented in Table-2.

Morphology and beads size
The shape and surface morphological examination and beads size were evaluated by the previous method.

In vitro floating study
In vitro floating study was performed using a USP-24 dissolution apparatus II containing 900 ml of simulated gastric fluid (SGF) of pH 1.2. The percentage of floating was measured by the visual observation.

Determination of drug loading and encapsulation efficiency
Accurately weighed (100 mg) grounded powder of beads and was soaked in 100 ml phosphate buffer (pH 7.5) and allowed to disintegrate completely for 4 hr. The drug loading and encapsulation efficiency from the resulting dispersion was determined by the reported method.

Table 1. Composition of the drug loaded polymeric blended pectin gum bead

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (C)</th>
<th>Gum</th>
<th>Oil</th>
<th>Calcium carbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>COB (HOB)</td>
<td>% w/v</td>
<td>% w/w</td>
<td>% w/v</td>
<td>% w/v</td>
</tr>
<tr>
<td>F₁</td>
<td>C₁</td>
<td>0.70</td>
<td>1.5:1</td>
<td>05</td>
</tr>
<tr>
<td>F₂</td>
<td>C₂</td>
<td>0.70</td>
<td>1.5:1</td>
<td>10</td>
</tr>
<tr>
<td>F₃</td>
<td>C₃</td>
<td>0.70</td>
<td>1.5:1</td>
<td>15</td>
</tr>
<tr>
<td>F₄</td>
<td>C₄</td>
<td>0.70</td>
<td>1.5:1</td>
<td>05</td>
</tr>
<tr>
<td>F₅</td>
<td>C₅</td>
<td>0.70</td>
<td>2:0:0.5</td>
<td>05</td>
</tr>
<tr>
<td>F₆</td>
<td>C₆</td>
<td>0.70</td>
<td>2:0:0.5</td>
<td>10</td>
</tr>
<tr>
<td>F₇</td>
<td>C₇</td>
<td>0.70</td>
<td>2:0:0.5</td>
<td>15</td>
</tr>
<tr>
<td>F₈</td>
<td>C₈</td>
<td>0.70</td>
<td>2:0:0.5</td>
<td>15</td>
</tr>
</tbody>
</table>

Gum = pectin: carbopol / hydroxypropyl methylcellulose (HPMC)
COB = pectin: carbopol blend formulation
HOB = pectin: HPMC blended formulation
Oil = mineral oil

Table 2. Independent variables of the formulation bead coated with ethyl cellulose.

<table>
<thead>
<tr>
<th>Formulation (code)</th>
<th>EC-concentration ( % (w/v ) )</th>
<th>Time of coating (min.)</th>
<th>% Drug release t₄₈₀(min)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₁₅</td>
<td>5</td>
<td>5</td>
<td>76 ± 1.7</td>
<td>0.982</td>
</tr>
<tr>
<td>F₂₅</td>
<td>5</td>
<td>10</td>
<td>65±1.6</td>
<td>0.964</td>
</tr>
<tr>
<td>F₃₅</td>
<td>10</td>
<td>5</td>
<td>60±1.5</td>
<td>0.923</td>
</tr>
</tbody>
</table>

EC = ethyl cellulose
R² = correlation coefficient, derived from zero order drug release kinetic
s = mean ± SD (n = 3)
F₁₅, F₂₅, F₃₅ = EC-coated optimized formulation of batch F₅.
In vitro drug release

In vitro dissolution studies were performed for all the formulation gel beads using USP 24 dissolution test apparatus II with a basket type. An accurately weighed 50 mg amount of the beads were taken in to 900 ml dissolution medium of the simulated gastric fluid (fasting state condition, pH of 1.2,) or phthalate buffer solution, (fed state condition, pH of 3.4) and the drug assayed by the previous method. Additionally, an experimental batch BE and BF containing 10 mg clarithromycin and lactose (q.s.) filled in a capsule (#2) was used as a reference formulation.

Evaluation of concentration of the drug in gastric mucosa

Albino rats were fasted for 8 hr and then divided into five groups of three animals. The protocol of the study was approved by animal ethical committee of the department. The animals were treated by an intraperitoneal injection of omeprazole at the dose of 15 mg/Kg to suppress gastric acid secretion. After one hour of omeprazole treatment, one group was treated with Cl plain and other groups were treated with formulation batch F<sub>5</sub> and EC-coated formulation (F<sub>15</sub>, F<sub>25</sub> and F<sub>35</sub>) at the equivalent dose of 10 mg/Kg. The drug concentration in the gastric mucosa was determined by the published method.

In vitro growth inhibition study

The protocol of the study was approved by animal ethical committee of the department. To study the effect of formulations on H. pylori growth inhibition, 10 ml of nutrient broth containing H. pylori were transferred into sterile test tubes. Plain drug (Cl), batch F<sub>5</sub> and EC-coated formulation were taken containing Cl equivalent to 20 µg/ml which is twice in concentration with respect to MIC (10 µg/ml ) and added to the tubes and all the tubes were incubated at 37°C in a microaerobic atmosphere for 24 hr. The optical density was determined to assess growth inhibition of the bacteria by our previous work.

In vivo H. pylori eradication study

The animals were divided in to five groups of six animals. The animals were inoculated orally with one ml culture broth after fasting for 24 hr and each dose was contained 10<sup>9</sup> CFU of H. pylori. Fourteen days after infection, clarithromycin suspension (dispersed well in 0.5% w/v of methylcellulose solution) and the EC coated formulation was orally administered once a day for three consecutive days at a dose of 10 mg/kg. The blank microbeads were also administered in the same manner as control. Three days after administration of the final dose, the Mongolian gerbils were killed and the stomachs were removed. Each stomach was homogenized with brucella broth (3 ml/stomach), and serial dilutions were plated on modified Skirrow’s medium. The agar plates were incubated for 4 days at 37°C under microaerobic conditions in GasPak (BD Diagnostic Systems, Sparks, MD). The viable cell counts for each stomach were calculated by counting the number of colonies on the agar plates. The colonies were identified as H. pylori by morphology and urease activity. The number of colonies per plate was counted and expressed as log CFU per gastric wall.

Statistical analysis

The results are expressed as mean ± SD. Statistically significant differences between groups were defined as p < 0.05.

Results and Discussion

Preparation and optimization of microgel beads

Microbeads were design with 2<sup>3</sup> factorial patterns wherein pectin helped to emulsify the mixture of water and oil phase during the homogenization process. Nonenteric polymer ethyl cellulose was selected for coating of gel beads due to its stability in gastric pH and based on the reports on the use of ethyl cellulose for coating on floating micro particles to modify the drug release.

Morphology and beads size

HOB formulation of batch C<sub>5</sub> and the COB of batch F<sub>5</sub> are shown in Figure 2 a) and Figure 2 b) respectively. The beads of batch F<sub>5</sub> were white, spherical and presence of minor projections may be attributed to presence of insoluble drug particles in the bead matrix, while that of batch C<sub>5</sub> were smooth translucent and less spherical.
Particle size of HOB formulation was found 0.84±0.3 mm and 1.12±0.8 mm while that of COB formulation was found 0.95±0.6 mm and 1.30±0.2 mm (Table 1). The pattern of particle size distribution of formulated beads is shown in figure 3. It has been observed that the diameter of beads increased significantly (p < 0.05) by increasing polymer and calcium carbonate concentration. The effects of polymer and calcium carbonate were similar with our previous study.\(^\text{13}\)

In vitro floating study
The floating efficacy of batch F\(_5\) and C\(_5\) was found to be 94±1.6 % and 90±1.3 % respectively. Upon contact with acidic medium, in situ pores on the entire surface of microbeads were produced due to leaching of Ca\(^{2+}\) ions consequently to provide buoyant formulation. On increasing CaCO\(_3\) concentration, buoyancy of the beads was increased. The increase in amount of Ca\(^{2+}\) ions and subsequently evolved CO\(_2\) were entrapped in the gel network of the formulation consequently rises the beads in the floating medium or the stomach.

![Figure 2. Scanning electron micrograph (SEM) of floating microbeads of clarithromycin. SEM of prepared microspheres of batch F\(_5\), (a). Surface morphology of prepared beads of batch C\(_5\), (b).](image)

![Figure 3. Particle size distribution pattern of prepared microgel beads formulation. Bars represent mean ± S.D. (n = 3).](image)
Determination of drug loading and encapsulation efficiency

F_5_ gel bead of COF formulation was found to be highest drug loading (60±1.8 % w/w) while that of HOB of batch C_2 was found to be minimum drug loading (37±1.2 % w/w). This was due to formation of comparative highly Ca_{2+} ions entrapped viscous HPMC blend dispersion and was less entrapped clarithromycin as carbopol blend formulation. It has been observed that no significance effect was found by increment of the polymer and CaCO_3 concentration. Encapsulation efficiency of COB formulation was varied between 88 ± 0.6% to 70± 0.5 % while that of HOB was between 80± 0.4% to 73±0.8 %. Encapsulation efficiency was decreased as the oil concentration in the formulation was increased. Furthermore, no significant (p > 0.05) effect was found for CaCO_3 and CaCl_2 on loading of the drug.

In vitro drug release

The gel beads were exhibited a biphasic Cl release pattern as an initial rapid drug release phase and was followed to the decline phase up to 8 hr of the study (Figure 4). The drug release from batch F_5_(empty state) and batch F_5_F(fed state) was found to be 65.4 ± 2.10 % and 60.0 ± 1.5 % respectively while that of batch C_5_ (empty state) and batch C_5_F(fed state) was found to be 59±1.2% and 54±1.6% respectively. The drug release from formulation COB was found to be fast while that of formulation HOB was relatively slow, this was evidenced that carbopol blend in the polymer composite was produced low viscosity of the polymer dispersion consequently smaller microgel beads and was shortened the diffusion path length for release of the drug.

The drug release from batch F_(empty state) was found to be 88±1.2% while that of batch F_F (fed state) was found to be 86.9± 1.6% up to 2 hrs of the study and could not sustain for 8 hrs. Drug release of the formulation batch F_5 followed the Higuchi (R^2 = 0.946, n = 0.37) and Pappas models (R^2 = 0.948, n = 0.34) respectively, suggested a diffusion based mechanism of the drug release as the diffusion exponent values were less than 0.45.

However, EC-coated batch F_{15} was found to be highest drug release efficiency (76±1.7%) for 8 hr of the study (Figure 5). Therefore, batch F_{15} was showed maximum dissolution efficiency and was best fitted in zero-order release kinetic (Table 2) and was recognising as an optimized formulation of the study.

### Table 3. Characteristics of prepared formulation of polymeric blended pectin gel beads.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Diameter (mm) a, b, c</th>
<th>Buoyancy (%) a, c</th>
<th>Encapsulation Efficiency % (w/w) a, c</th>
<th>Drug content % a, d, c</th>
</tr>
</thead>
<tbody>
<tr>
<td>COB HOB</td>
<td>COB</td>
<td>HOB</td>
<td>COB</td>
<td>HOB</td>
</tr>
<tr>
<td>F_1 C_1</td>
<td>0.95±0.6</td>
<td>0.84±0.3</td>
<td>85±1.2</td>
<td>76±1.3</td>
</tr>
<tr>
<td>F_2 C_2</td>
<td>1.0±0.2</td>
<td>0.92±0.4</td>
<td>82±1.3</td>
<td>73±1.5</td>
</tr>
<tr>
<td>F_3 C_3</td>
<td>1.27±0.4</td>
<td>0.98±0.5</td>
<td>77±1.5</td>
<td>68±1.6</td>
</tr>
<tr>
<td>F_4 C_4</td>
<td>0.98±0.3</td>
<td>0.89±0.4</td>
<td>74±1.2</td>
<td>70±1.4</td>
</tr>
<tr>
<td>F_5 C_5</td>
<td>1.18±0.5</td>
<td>1.0±0.2</td>
<td>94±1.6</td>
<td>80±0.6</td>
</tr>
<tr>
<td>F_6 C_6</td>
<td>1.30±0.2</td>
<td>1.12±0.8</td>
<td>66±1.8</td>
<td>63±1.4</td>
</tr>
<tr>
<td>F_7 C_7</td>
<td>0.98±0.6</td>
<td>0.97±0.3</td>
<td>61±1.2</td>
<td>60±1.2</td>
</tr>
<tr>
<td>F_8 C_8</td>
<td>1.13±0.3</td>
<td>1.1±0.5</td>
<td>69±1.6</td>
<td>63±1.3</td>
</tr>
</tbody>
</table>

COB = pectin: carbopol blended formulation.
HOB = pectin: hydroxypropyl methylcellulose (HPMC).
a = Mean ± SD
b = n = 20
c = Mean ± SD (n = 3)
d = Drug content in each 100 mg of beads.
Figure 4. In vitro comparative drug release pattern in empty and fed state condition. Bars represent mean ± S.D. (n = 3).

Evaluation of the drug concentration in the gastric mucosa
The drug concentration in gastric mucosa of the experimental animal was evaluated in order to assess availability of the drug exclusive to the infected mucosal layer. The drug concentration in the gastric mucosa was found to be 50±0.02 μg/ml (batch F₁), 60±0.05 μg/ml (batch F₁₅), 57±0.02 μg/ml (batch F₂₅), 55±0.04 μg/ml (batch F₃₅) and 20±0.06 μg/ml (batch Cl) up to one hour and was gradually decreased (Figure 6). However, highest drug concentration in mucosa was showed by batch F₁₅ and was attributed to comparative more partition of the drug from the formulation barrier in to gastric mucosa cells.

Figure 5. Comparative in vitro drug release pattern of ethyl cellulose coated micro bead. Bars represent mean ± S.D. (n = 3).
**In vitro growth inhibition study**

The effect of antimicrobial activity of the formulation and plain drug was investigated in *H. pylori* at various time intervals for 12 hr (Figure 7). The *H. pylori* culture tubes containing Cl free microbeads did not show significant growth inhibition (2.5 ±1.2 %) at the end of 12 hr of incubation, which suggested that ingredients which are used in formulation have no antimicrobial activity. The percentage growth inhibition value of batch F5 was found to be 77 ± 1.6 % while that of batch F15 and batch F35 were found 100 % and 95 ±1.8% respectively , after incubation for 8 hr. The results clearly indicate that the microbeads of batch F15 was showed good growth inhibition in *vitro* culture and may provide the better treatment in *H. pylori* infection.

**In vivo *H. pylori* eradication study**

In the control group receiving no Cl, around $10^5$ viable bacteria colonized in the animal stomach. Control batch showed negligible bacterial clearance while that of clarithromycin suspension therapy showed 33% bacterial clearance at the dose of 10 mg/kg (Table 4).This effect could be attributed to short residence time of Cl suspension in the stomach and the concentration of Cl was not sufficient in gastric mucus layer to eradicate *H. pylori*. EC- coated formulation batch F15 and batch F25 were showed 100 % bacterial clearance while that of batch F35 was showed 77 % clearance of the bacterial colony. This is because of the longer residence time of batch F15 and batch F25 in the stomach, which enabled high concentration of clarithromycin to reach the bacteria underlying the gastric mucosal layer.
Table 4. Effect of different formulations against gastric infection caused by *Helicobacter pylori* in Mongolian gerbils after oral administration.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Dose (mg/Kg)a</th>
<th>Clearance rate (no. of gerbils cleared infection)/total no. (%)</th>
<th>Bacterial recovery (log CFU/stomach)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0/6(0)</td>
<td>8.46 ± 0.26</td>
</tr>
<tr>
<td>Cl suspension</td>
<td>10</td>
<td>2/6(33)</td>
<td>4.08± 0.14</td>
</tr>
<tr>
<td><em>Batch F₁₅</em></td>
<td>10</td>
<td>6/6(100)</td>
<td>ND</td>
</tr>
<tr>
<td><em>Batch F₂₅</em></td>
<td>10</td>
<td>5/6(100)</td>
<td>3.41± 0.12</td>
</tr>
<tr>
<td><em>Batch F₃₅</em></td>
<td>10</td>
<td>4/6(77)</td>
<td>2.02± 0.34</td>
</tr>
</tbody>
</table>

CFU = colony forming unit  
ND = colony not detected  
Cl = clarithromycin

a The given dose of clarithromycin was administered once daily for 3 days (batch F₁₅, F₂₅ and F₃₅ contains equivalent amount of Cl).  
b Bacterial cell counts less than $10^{1.45}$ CFU were considered to be $10^{1.45}$ to calculate the mean. Values are means ± S.E.

*F₁₅, F₂₅, F₃₅* are ethyl cellulose coated formulation, prepared by coating of selected batch F₂.

**Conclusion**

The designed, optimized gel beads of clarithromycin were formulated to provide sustained release of drug with view to providing an effective and safe therapy for *H. pylori* infection. The formulation batch F₁₅ has feasibility of targeting in stomach and sustaining the drug release from the gel beads over the period of at least 8 hr. Further, the prepared ethylcellulose coated beads were effective in cleaning *H. pylori* in the infected Gerbil's stomach than the plain clarithromycine suspension, which is important from the viewpoint of reducing adverse effect during the therapy. Therefore, pH sensitive gastroretentive microbeads of clarithromycin may be better for treating gastric *H. pylori* infection.

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**Conflict of Interest**

The authors associated with the study have strictly declared that they have no conflict of interest.

**Contribution of Authors**

We declare that this work was done by the authors (Girish Kumar Tripathi and Satyawan Singh) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Mr. Girish Kumar Tripathi was conceived and design protocol of the study and further the study was improved by Dr. Satyawan Singh. The Tripathi was also involved in analysis of the study data.

**References**


