Abstract

A gastro retentive pH sensitive system has been a frontier approach to release the drug in controlled manner in stomach and duodenum. The aim of the study is to develop reliable formulation of amoxicillin which will release the drug in controlled way at specific site with acidic pH stimulus present in the gastric region. In the present investigation pectin based oil entrapped micro gel beads were prepared by ionic gelation technique using castor oil and mineral oil. The developed beads were evaluated in term of diameter, surface morphology, floating lag time, encapsulation efficiency, in vitro drug release. Prepared microbeads were regular and spherical in shape. The formulation exhibited sustained release profile and was best fitted in the Peppas model with $n < 0.45$. Subsequent coating of microbeads exhibited zero-order sustained pattern of amoxicillin release up to 8 hrs.

The Results provides evidence that optimized gel bead may be used to incorporate antibiotics like amoxicillin and may be effective when administered locally in the stomach to cure microbial infection.

Keywords: Amoxicillin (Am); Calcium pectinate bead; Residential time; pH Sensitive; Ethyl cellulose (EC);

Introduction

Oral route of administration still remains the route of choice for the majority of clinical application for the local action in the gastrointestinal tract (GI). Recent scientific report and patent literature reveals, interest in novel dosage forms that can be retained in the stomach for a prolonged and predictable period of time. The feasible approaches for achieving a prolonged and predictable drug delivery are control the gastric residence time (GRT), using gastric retentive dosage form that can provide newer therapeutic option[1, 2]. Floating drug and bioadhesive drug delivery systems are widely used techniques for gastrentention [3, 4]. Floating drug delivery systems have a bulk density lower than gastric fluids and thus remain buoyant in the stomach for a prolonged period of time, without affecting the gastric emptying rate. While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the system and remaining part of the residual system is slowly emptied from the stomach and enhanced GRT of the drug [5-7].

This study was envisaged to develop mucosaadhesive gastric retentive formulation of amoxicillin in order to achieve targeted release of...
amoxicillin in acidic pH of the gastrointestinal tract. The concept of multiunit gastro retentive microbead can be utilized to provide a more reliable and long lasting release of drug in the stomach for local and systemic action. Narkar et al. [8] prepared and evaluated the oral mucoadhesive beads of amoxicillin. Babu et al. [9] prepared gellan gum macro beads of amoxicillin. The oil entrapped pectin gel beads were found to be a potential candidate for targeted drug delivery and are anticipated to be useful in the treatment of microbial infection in the gastric region [10].

Gastric retentive and floating approaches were undertaken to develop reliable formulation of amoxicillin that has all the advantages to modify the release of the drug with continuous and for prolonged period. Thus the formulations was uniform distributed in gastric site and maintain long time minimum inhibitory concentration (MIC) of the drug around microbial cell line [11]. Amoxicillin is semi-synthetic amino penicillin with a broad-spectrum bactericidal activity [12, 13]. Pectin is colloidal polygalacturonic acid in which some of the carboxylic group is esterified with methyl group [14, 15]. Pectin can reduce interfacial tension between oil and a water phase and is efficient for the preparation of emulsion [14]. In the present investigation, the amoxicillin –loaded gastroretantive emulsion gel beads of calcium pectinate were developed and investigated with the aim to achieve a gastroretentive, multiple units, and controlled release formulation of amoxicillin.

Materials and methods

**Materials**

Amoxicillin was obtained as gift sample from, Ranboxy laboratory Devash, India. Low methoxy pectin with the degree of esterification of 35% and ethyl cellulose were obtained from S.D. Fine Chem. India. Light mineral oil and castor oil were obtained from the Central Drug House, Ind.

**Method**

**Preparation of calcium pectinate beads**

The gel beads were formulated with $2^3$ factorial design patterns (Table 1). The effect of concentration of the oils (castor and mineral oils), pectin and calcium chloride ($CaCl_2$) were selected as independent variable. Effect of the dependent variables in the formulation was investigated in terms of bead diameter, floating lag time, encapsulation efficiency and drug content.

**Table 1: Composition of drug loaded calcium pectinate gel beads**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug</th>
<th>Gum</th>
<th>Oil</th>
<th>Calcium chloride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castor oil (COB)</td>
<td>Mineral oil (MOB)</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>S1</td>
<td>P1</td>
<td>0.65</td>
<td>1.50</td>
<td>05</td>
</tr>
<tr>
<td>S2</td>
<td>P2</td>
<td>0.65</td>
<td>1.50</td>
<td>10</td>
</tr>
<tr>
<td>S3</td>
<td>P3</td>
<td>0.65</td>
<td>2.10</td>
<td>05</td>
</tr>
<tr>
<td>S4</td>
<td>P4</td>
<td>0.65</td>
<td>1.50</td>
<td>05</td>
</tr>
<tr>
<td>S5</td>
<td>P5</td>
<td>0.65</td>
<td>1.50</td>
<td>15</td>
</tr>
<tr>
<td>S6</td>
<td>P6</td>
<td>0.65</td>
<td>2.10</td>
<td>05</td>
</tr>
<tr>
<td>S7</td>
<td>P7</td>
<td>0.65</td>
<td>1.50</td>
<td>15</td>
</tr>
<tr>
<td>S8</td>
<td>P8</td>
<td>0.65</td>
<td>2.10</td>
<td>10</td>
</tr>
<tr>
<td>S9</td>
<td>P9</td>
<td>0.65</td>
<td>2.10</td>
<td>15</td>
</tr>
</tbody>
</table>

COB = castor oil entrapped formulation
MOB = mineral oil entrapped formulation

Oil entrapped calcium pectinate gel beads were prepared by ionic gelation method. A 0.65 % w/v of drug was dispersed in varying concentrations (1.50 - 2.10 % w/v) of aqueous solution of pectin with continuous stirring until a uniform dispersion was obtained. The mixture was emulsified with either mineral oil or castor oil using Silverson emulsifier (Hicon, India) stirred at 500 rpm for 5 min. The resultant drug loaded emulsions was dropped through a 21G syringe needle separately into 100 ml of 0.275- 0.45 mol ml$^{-1}$ of calcium chloride solution, maintained under gentle agitation to improve the mechanical strength of the beads and also to prevent aggregation of the formed beads. Amoxicillin loaded beads containing either castor oil (COB) or mineral oil (MOB) were
separated, washed with water and allowed to dry at 40 °C in side tray dryer for 6 hrs.

**Particle size and morphology**

Particle size of the prepared beads were determined in three set using an optical microscope (Model BH-2, Olympus, Japan) fitted with a stage and an ocular micrometer. Mean diameter was calculated by measuring diameter of 20 dried beads. The external and internal morphology of micro gel beads were studied by scanning electron microscopy. The micro beads were coated with gold palladium under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The coated samples were then observed with a scanning electron microscope.

**In vitro floating studies**

The *in vitro* floating study was performed using a USP 24 dissolution apparatus II, having 500 ml of phthalate buffer solution (pH 3.4). The medium temperature was kept at 37 ± 0.5 °C. The floating beads (1.0 g beads) were soaked in the dissolution medium and the medium was agitated with a paddle at 50 rpm. After agitation, the beads that floated on the surface of the medium and those that settled down at the bottom of the flask were recovered separately [15].

**Determination of drug loading and encapsulation efficiency**

Accurately weighed (100 mg) grounded powder of beads was soaked in 100 ml phosphate buffer (pH 7.5) and allowed to disintegrates completely for 4 h (16). The resulting dispersion was sonicated using a probe sonicator (UP 400 s, Dr. Hielscher GmbH, Germany) for 30 min and then filtered through a 0.45 μm filter. The polymeric debris was washed twice with fresh phosphate buffer to extract any adhered drug and drug content was determined spectrophotometrically at 334.5 nm against constructed a calibration curve. The encapsulation efficiency (EE) was calculated according to the relationship in Eq 1

$$EE\ (%) = \frac{(C/T) \times 100}{\%} \quad (1)$$

Where C is the calculated drug content and T is the theoretical drug content

**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 formulation dropped in 900 ml of both fasted state (simulated gastric fluid, SGF, pH 1.2) and fed state (phthalate buffer solution, pH 3.4) conditions maintained at a temperature of 37°C ± 0.5°C and stirred at a speed of 50 rpm. At different time intervals, a 10 - ml aliquot of the sample was withdrawn at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 hrs and the volume was replaced with an equivalent amount of plain dissolution medium. The collected samples were filtered and suitably diluted and analyzed at 334.5 nm using a UV-visible spectrophotometer (Shimadzu). Drug release data were corrected for the values of the drug loss. Additionally, an experimental batch BF and BE containing 10 mg amoxicillin and lactose (q.s.) filled in a capsule [# 2] was used as a reference formulation. Drug release data were corrected for the values of the drug loss during sampling [17].

**Gastric residence efficacy**

Gastric residence efficacy was evaluated by the method of Zheng et al. [18] with slight modification. Albino rates selected for the study were fasted for 8 hrs and then divided into two groups and each group consisted of three animals. Each group was pre-treated by an intraperitoneal injection of omeprazole at a 15 mg/Kg dose to suppress gastric acid secretion. After 1 h of the administration of the drug each group was given a single oral dose of the gel beads 500 bead (batch P5 and S5) aqueous suspension. After 1, 4, and 8 hrs, the rate was scarified by cervical dislocation. The stomachs were removed. The microbeads that retained in the stomach were counted and the percentage of the remaining beads was calculated.
Coating of gel beads

The prepared formulation batch S₅ was selected for optimization due to higher value of evaluations parameters like particle size, lag time, encapsulation efficiency, drug content and gastric residence time. The selected beads were coated with EC in 2² factorial designs (as table 2.). The coating material taken as 5 - 10% w/v ethyl cellulose (EC) solution in acetone and coating time 5 - 10 min. The gel beads (2 g) were placed in a fluidized bed dryer (TG 100, Retsch, Germany) and the fluidized beads were sprayed with the coating solution for a period of 10 min at an air inlet speed of 220 m s⁻¹ at room temperature. The beads were dried at room temperature for a period of 24 hrs until the solvent was evaporated, leaving a film coat on the gel beads.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>EC concentration % m/v</th>
<th>Time of coating (min)</th>
<th>DR₄₈₀ hr (%)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>S₁₅</td>
<td>5</td>
<td>5</td>
<td>85.7</td>
<td>0.991</td>
</tr>
<tr>
<td>S₂₅</td>
<td>5</td>
<td>10</td>
<td>69.8</td>
<td>0.980</td>
</tr>
<tr>
<td>S₃₅</td>
<td>10</td>
<td>5</td>
<td>64.4</td>
<td>0.972</td>
</tr>
<tr>
<td>S₄₅</td>
<td>10</td>
<td>10</td>
<td>59.2</td>
<td>0.971</td>
</tr>
</tbody>
</table>

DR = dissolution efficiency  
EC = ethylcellulose concentration  
R² = correlation coefficient, derived from zero order drug release kinetic.
S₁₅, S₂₅, S₃₅ and S₄₅ = optimized ethylcellulose coated formulation of batch S₅ batch.

Table 3: Characterization of prepared formulation of calcium pectinate gel beads

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Diameter (mm)ᵃ,ᵇ,ᶜ</th>
<th>Lag time (sec)ᵈ,ᵉ</th>
<th>Encapsulation efficiency (%w/w)ᶠ,ᵍ</th>
<th>Drug content %ᵃ,ᵈ,ᵉ</th>
</tr>
</thead>
<tbody>
<tr>
<td>COB</td>
<td>MOB</td>
<td>MOB</td>
<td>COB</td>
<td>MOB</td>
</tr>
<tr>
<td>S₁</td>
<td>P₁</td>
<td>1.54±0.8</td>
<td>1.74±0.3</td>
<td>24±1.3</td>
</tr>
<tr>
<td>S₂</td>
<td>P₂</td>
<td>1.62±0.7</td>
<td>1.83±0.5</td>
<td>32±1.5</td>
</tr>
<tr>
<td>S₃</td>
<td>P₃</td>
<td>1.66±0.5</td>
<td>1.88±0.4</td>
<td>50±1.3</td>
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<tr>
<td>S₄</td>
<td>P₄</td>
<td>1.58±0.3</td>
<td>1.79±0.5</td>
<td>19±1.9</td>
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<tr>
<td>S₅</td>
<td>P₅</td>
<td>1.64±0.3</td>
<td>1.75±0.7</td>
<td>14±1.4</td>
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<tr>
<td>S₆</td>
<td>P₆</td>
<td>1.76±0.2</td>
<td>1.87±0.6</td>
<td>20±1.7</td>
</tr>
<tr>
<td>S₇</td>
<td>P₇</td>
<td>1.68±0.4</td>
<td>1.89±0.5</td>
<td>43±1.8</td>
</tr>
<tr>
<td>S₈</td>
<td>P₈</td>
<td>1.69±0.3</td>
<td>1.90±0.6</td>
<td>46±1.5</td>
</tr>
</tbody>
</table>

Statistical analysis

The experimental results were expressed as mean ± SD (standard deviation). Statistical evaluation of data was performed using an analysis of variance (ANOVA) and, depending on the outcome of the ANOVA (Dunnett’s multiple comparison test). The evaluation data was used to assess the significance of differences. Statistically significant difference between the means of batches were defined as P<0.05.

Result and discussion

Morphology of microgel bead

Gel micro beads were produce due to gelation and cross linking of Ca⁺⁺ ions, provided a gel barrier at the surface of the formulation. Pectin helped to emulsify the mixture of water and oil phase during the homogenization process and its emulsion stabilization property could be explained by its surface-active ability to reduce the interfacial tension between the oil and water phases. Scanning electron micrograph (SEM) of amoxicillin loaded microgel bead and their surface morphology are shown in Figures 1A & 1B. Gel beads prepared from mineral oil (MOB) were white, translucent and elastic, whereas castor oil entrapped gel beads (COB) were off-white translucent and rigid in texture.
Particle Size of bead

The effect of various formulation parameters on the particle size of prepared floating beads are shown in Table 3. The diameter of MOB formulation vary in size 1.54±0.8 to 1.76±0.2 mm and COB formulation size was ranges 1.74±0.3 to 2.10 ±0.4 mm . Size distribution pattern of micro beads of both the formulation was found significance difference (Figure 2). It has been found that the diameter of beads increased significantly (P< 0.05) on increasing concentration of the polymer . This could be due to increase in micro-viscosity of the polymeric dispersion eventually resulted into formation of bigger beads. Large size beads were form as concentration of CaCl2 increased, this is due to generation of excess Ca²⁺ and resulted to formation of weaken and flexible large gel beads.

Lag time and buoyancy

The time taken for the beads to float at the surface of the medium known as floating lag time. The lag time was found to vary 14-57 sec. (MOB formulation) and 17-66 sec. (COB formulation). The minimum lag time was found in MOB formulation (14±1.4 sec batch P5) and COB formulation was 17±1.8 sec. (in batch S5). Buoyancy is an important characteristic in sustained drug delivery. On increasing CaCl₂ concentration, floating lag time was reduced. The increase in the amount of Ca²⁺ and consequently the amount of CO₂, evolved are responsible for the observed reduction in floating lag time. Floating lag time was increased as concentration of the oil in the formulation increase, this could be due to large number of oil globules tend to stick each other and produce flocculation or they may unite and produced coalescence in the large droplet form. Droplets may fill in the pores of the system, resulted in decrease porosity of the bead.

Encapsulation efficiency and drug content

Encapsulation of the drug was found to be consistently higher in the formulation COB of batch S5 (77±0.2%) and MOB formulation of batch P5 (65±0.6 %). The drug contents were 61± 1.14 % and 56±1.46% in batch S5 and batch P5. The encapsulation efficiency increased progressively with increasing polymer concentration .This is because of the availability of excess of the polymer resulted in formation of larger size of beads entrapping more amount of the drug.

Gastric residence efficacy

Gastric residence studies were carried out to ensure the transit of design formulation in the mucosa. Profile of the gastric residence time of microbead is shown in Figure 3. Gastric retention of selected batch S5 and batch P5 was found 59±1.6 % and 54±1.2 % respectively at 8 hrs of the study .Significance difference (P < 0.05) of gastric residence of MOB (batch P5) and COB(batch S5) formulations was due to affinity of the formulation towards glycoproteins of the
mucus layer of stomach. The formulation was floated in the stomach and later adhered to the mucous layer so both mechanisms enhanced the gastric residence time.

Figure 3: Gastric residence pattern in albino rat of batches S₅ and P₅

**In Vitro drug release studies**

*In vitro* amoxicillin release study of gel beads was carried out in the fasted state (SGF solution of pH 1.2), and in the fed state (phthalate buffer of pH 3.4) for a period of 8 hrs. Gel beads exhibited a biphasic amoxicillin release profile as an initial rapid drug release phase (burst effect) was followed by a slower, gradually declining drug release phase was extended up to 8 hrs (Figure 4A.). The drug release from experimental reference (capsule was filled with 10 mg of amoxicillin) and the release study was 90.09 ± 2.48 % (batch BE) in empty state and 87.5±2.45% (batch BF) in fed state in to within 2 hrs and could not sustain the release of the drug over 8 hrs. High Release of amoxicillin was found in batch S₅ (59 ± 2.14 %) and batch M₆ (38± 2.43 %) in fed of 8 hrs of the study. Various release kinetic models (zero order, Higuchi and Korshmaer-Peppas model) were applied for the elucidation of mechanism of the drug release from floating gel beads in the fed state condition[20-22]. Drug release from the optimized formulations S₅ followed the Higuchi (R² = 0.943) and Peppas models (R² = 0.986, n = 0.36), suggested a diffusion based mechanism of drug release as the diffusion exponent values were less than 0.45 [19].

Non enteric 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 [19]. The desirable dependent response showed zero-order release pattern from EC-coated formulation in the fed state. The release of amoxicillin from formulation S₁₅ gel beads was highest 85.7 ± 1.2% at end of 8 hrs of study (Figure 4A). The formulation batch S₁₅ exhibited maximum dissolution efficiency was best fitted zero-order release and R² value of 0.9991 (Table 2) was optimized batch of the controlled-release formulation of amoxicillin.

**Conclusion**

Based on the results of this study, it reveals that optimized ethylcellulose coated batch S₁₅ micro gel beads of amoxicillin have pH sensitive controlled release of drug in acidic region of the gastrointestinal tract. Therefore, it may be capable of delivering amoxicillin to stomach sites, thus opening up the possibility of targeting the drug to gastric sites for the cure of microbial infections such as that caused by *H. pylori*.

**List of abbreviations**

Am, amoxicillin; EC, ethylcellulose; GI, gastrointestinal tract; MIC, minimum inhibitory concentration; COB, castor oil entrapped formulation; MOB, mineral oil entrapped formulation;

**Authors’ contributions**

Experimental and analysis of the study was performed by Girish Kumar Tripathi and Dr. Satyawan Singh given final approval of the work.

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