FORMULATION OF TETRANDRINE BEADS USING IONIC GELATION METHOD CA-PECTINATE COATED PH-SENSITIVE POLYMERS AS COLON-TARGETED DOSAGE FORM

RADITYA ISWANDANA1*, KURNIA SARI SETIO PUTRI2, CINDY ESPREANCELLY SANDIATA1, SISILIA TRIANI1, SANTI PURNA SAR1, JOSHITA DJAJADISA2TRA1

1Laboratory of Pharmaceutics and Pharmaceutical Technology Development, Faculty of Pharmacy, Universitas Indonesia, Depok 16424, Indonesia. 2Laboratory of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Indonesia, Depok 16424, Indonesia.

ABSTRACT

Objectives: Pectin, a natural polysaccharide, can be used as colon targeted drug delivery systems. Ionotropic gelation of pectin in the presence of certain divalent cations, such as calcium ions, protects drugs by producing insoluble hydrogels that can be used as a colon-targeted drug delivery carrier. In this study, calcium pectinate beads containing tetrandrine were made and were evaluated for in-vitro drug release and in-vivo study.

Methods: Calcium pectinate beads were prepared by ionic gelation method with varied calcium chloride concentration (5%, 10%, and 15%). The best formula was coated with pH sensitive polymers, i.e., Eudragit L100-55, Eudragit L100, hydroxypropyl methylcellulose phthalate HP-55 or cellulose acetate phthalate.

Results: Characterization results showed that the beads produced were quite spherical and had yellow-brownish color. After the coating process, beads were used in in-vitro drug release and targeted test. From in-vitro release study, beads coated with Eudragit L100 10% has shown good colon targeted dosage form with percent cumulative release 57.87%. This result also confirmed with the in-vivo test. Beads which were coated by Eudragit L100 10% could be found in the rat intestine.

Conclusion: Formula 1 (5% calcium chloride concentration) was chosen as the best beads characterization. Formula 1C (5% beads coated with 10% Eudragit L100) showed an optimal protection from gastric acid in the in-vitro release study and able to deliver the beads to the intestine in the in-vivo targeted test.

Keywords: Beads, Tetrandrine, Calcium pectinate, Ionic gelation, Colon-targeted.

INTRODUCTION

Natural polymer, such as pectin, provides a potential in pharmaceutical industry application. Pectin has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of drugs, proteins, and cells. Pectin had been widely used as a carrier to deliver drugs to the digestive tract, including tablet matrix, gel beads, and film-coated dosage form. Pectin can be used as colon-targeted drug preparations because it has a long retention in the gastrointestinal tract and a complete degradation by the living bacteria in the intestine. Besides that, pectin is nontoxic, biodegradable, and biocompatible [1].

Drugs delivery system to the colon should provide protection of the drugs during the delivery to the colon, which including prevent the release and drug absorption in the stomach and intestine, then the bio active release and absorption can happen in the colon [2]. Drugs delivery system to the colon has certain challenges because the colon is the furthest segment in the gastrointestinal tract and a colon-targeted formulation will be affected by various condition and environment during the delivery via the gastrointestinal tract, including the pH, enzyme, electrolyte, transit time, and pressure [3].

Drug formulation with pH sensitive polymer can protect the active substance from gastric acid and proximal of the small intestine. This pH-sensitive polymer can tolerate dissolution in the gastric acid environment; however, it can be dissolved in a higher pH in the intestine. This pH-sensitive polymer can be split in ileum terminal pH, thus providing a targeted drug delivery to the colon [4]. Several examples of pH-sensitive polymer which commonly used for colon targeted drug delivery were methacrylate polymer in acid-base, known as Eudragit.

Eudragit L100-55 and Eudragit L100 was used as pH sensitive coat to inhibit drug release in the gastric and targeting the release in the intestine [5]. Furthermore, hydroxypropyl methyl cellulosephthalate (HPMCP) HP-55 and cellulose acetate phthalate (CAP) can be used for this purpose.

At present, there are several antifibrotic compounds under research, such as galunisertib [6], rosmarinic acid [7], and tetrandrine. We used tetrandrine as an antifibrotic drug model. Tetrandrine was an alkaloid isolated from Stephania tetrandra S. Moore root, studied as an inhibitor working in transforming growth factor (TGF-β) signaling pathway (TGF-inhibitor) [8]. Therefore, it was expected to have potential in intestinal fibrosis treatment. Intestinal fibrosis was a common complication of inflammatory bowel disease and can happen in ulcerative colitis and Crohn’s disease (CD), but more often appear on CD. Fibrosis as the consequences of local chronic inflammation was indicated with an abnormal deposition in matrix extracellular which led to organ dysfunction [9].

In this study, we developed a colon-targeted preparation, such as tetrandrine beads, using ionotropic gelation method, with pectin as the polymer and Ca2+ as the crosslinker, and coating the beads using pH sensitive polymer, i.e., Eudragit L100-55, Eudragit L100, HPMCP HP-55, or CAP. The final beads then characterized and conducted a test to obtain the drug release profile.

MATERIALS AND METHODS

Materials
Tetrandrine (Shaanxi Ciyuan Biotech, China), pectin (Danisco, United States of America), calcium chloride (Merck, Germany), Eudragit L100-
Calcium pectinate beads preparation
Pectin in 5% concentration was dissolved in distilled water. Then, tetrabrate was dissolved in HCl 0.5 N. Pectin and tetrabrate solutions mixed and stirred until homogeneous. Then, the pectin solution which already contained tetrabrate was shed using syringe needle in 26 G size to the medium containing calcium chloride in 200 rpm stirring rate. Beads produced in calcium chloride were stored for 15 minutes. Then, the beads were separated from the solutions and rinsed with deionized water three times and dried at room temperature. All beads formulae can be seen in Table 1.

Beads coating process with pH-sensitive polymers
Eudragit L100-55 was mixed with plasticizer, and talc thus can be obtained a 10% and 12.5% Eudragit L100-55. The plasticizer used was triethyl citrate in 25% concentration of Eudragit L100-55 mass used [10]. All coating materials then dissolved in acetone:isopropanol (1:1). Beads which would be coated were added to Eudragit L100-55 solutions while stirred. In beads coating with Eudragit L100, the coating was performed with a similar method and condition with Eudragit L100-55 for beads coating.

For CAP, a 10% (w/v) and 15% (w/v) solution in acetone were used for coating and triethyl citrate (25%, w/w) was used as a plasticizer. In the case of coating with HPMCP, a 10% (w/v) and 12% (w/v) solution in acetone were used and triethyl citrate (20w5%, w/w) was used as a plasticizer. Coating formula can be seen in Table 2.

Beads characterizations
Shape
Beads surface shape were observed using an optical microscope.

Table 1: Calcium pectinate beads formulations

<table>
<thead>
<tr>
<th>Formula</th>
<th>Pectin (%, w/v)</th>
<th>Calcium chloride (%, w/v)</th>
<th>Pectin:Tetrabrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>1:1</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>10</td>
<td>1:1</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>15</td>
<td>1:1</td>
</tr>
</tbody>
</table>

Table 2: Beads coating formulations

<table>
<thead>
<tr>
<th>Formula</th>
<th>Polymer</th>
<th>Concentrations (%, w/v)</th>
<th>Plasticizer concentrations (%, w/w)</th>
<th>Talc (%)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Eudragit L100-55</td>
<td>10</td>
<td>2.5</td>
<td>5</td>
<td>Acetone:isopropanol</td>
</tr>
<tr>
<td>B</td>
<td>Eudragit L100-55</td>
<td>12.5</td>
<td>3.125</td>
<td>6.25</td>
<td>(1:1)</td>
</tr>
<tr>
<td>C</td>
<td>Eudragit L100</td>
<td>10</td>
<td>2.5</td>
<td>5</td>
<td>Acetone</td>
</tr>
<tr>
<td>D</td>
<td>Eudragit L100</td>
<td>12.5</td>
<td>3.125</td>
<td>6.25</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>HPMCP HP-55</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>Acetone</td>
</tr>
<tr>
<td>F</td>
<td>HPMCP HP-55</td>
<td>12</td>
<td>25</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>CAP</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>CAP</td>
<td>15</td>
<td>25</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>
Japanese rat weighing 280 nm wavelength. Tetrandrine content was measured by comparing the calibration curve thus the tetrandrine entrapped could be measured.

Entrapment percentage (%E) obtained by comparing the core total content obtained with the total core theoretic, was measured using the following formula:

\[
\text{Entrapment efficiency (\%)} = \left( \frac{\text{Total core measured}}{\text{Total core theoretic}} \right) \times 100\%
\]

**Swelling test**

2.5 g beads sample from each formula was weighed (\(W_2\)) then placed on weighing dishes. 25 ml phosphate buffer pH 6.8 was added and stayed aside to expand in the room temperature. After 5 minutes, the sample was collected from the container, carefully dried and the rest of the medium was absorbed by filter paper, then weighed (\(W_1\)). After the sample had been weighed, they were put back into the medium. The weighing was following the same procedure which was performed in 5, 10, 15, 30, 60, 90, 120, and 180 minutes. Swelling ability was measured using the following formula:

\[
\text{Swelling ability (\%)} = \left( \frac{W_2 - W_1}{W_1} \right) \times 100\%
\]

**In-vitro release study**

The in-vitro release study was performed in hydrochloride acid 0.1 N pH 1.2, phosphate buffer pH 7.4, and phosphate buffer pH 6.8 media. Media volume used was 200.0 ml in 37±0.5°C using magnetic stirrer in 100 rpm rate. The drug release time in chloride acid 0.1 N pH 1.2 medium was observed for 2 hr, in phosphate buffer pH 7.4 medium was observed in 3 hrs, and the phosphate buffer pH 6.8 medium was observed in 3 hrs. 100 ml beads were weighed and put in the filter bag then put into the dissolution medium. 10 ml sample was collected, then the collected sample solution was immediately replaced with the same medium in some certain times. The absorption of the sample then measured using spectrophotometer UV-visible.

Measurement of the substance contained in the sample at n-minute was measured using the following formula:

\[
n_{\text{minute}} = \frac{y_{n}-a}{b \times 1000} + \cdots + \frac{y_{15}-a}{b \times 1000}
\]

Where:
- \(y\) = tetrandrine absorption
- \(y_n\) = tetrandrine absorption in n minute
- \(x\) = tetrandrine concentration
- \(fp\) = dissolution factor
- \(M\) = volume of release medium
- \(S\) = sampling volume
- \(a\) = intercept coefficient
- \(b\) = slope.

**In-vivo targeted test**

The in-vivo targeted test was performed qualitatively to define the beads toleration against gastric and proximal intestine pH thus could reach the colon. The test was performed in the Sprague-Dawley male rats with a weight of 260-330 g. Before performing the test, we conducted time orientation of the dissection. Beads were mixed in ±5.0 ml water and injected into the rats using gastric injection with the orientation result, and the colon condition was observed. Drugs targeted test was said successful if the beads found attached to the intestine. The experiments were approved by the Ethical Committee of Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia with ethical approval Reg. No. 319/UN2.F1/ETIK/2016.

**RESULTS AND DISCUSSION**

**Calcium pectinate beads**

Proportion was performed with 5% pectin solution and the concentration of calcium chloride (5%, 10%, and 15%) with tetrandrine and pectin ratio used was 1:1. Pectin in 5% concentration was dissolved in deionized water. Then, tetrandrine was dissolved in HQ 0.5 N. Pectin and tetrandrine solutions were mixed and stirred until homogeneous using stirrer. Then, the pectin solutions that had contained tetrandrine were shed using syringe needle in 2.6 G size into medium containing calcium chloride on 200 rpm stirring rate. When the pectin and tetrandrine mixtures were shed on calcium chloride, beads would immediately have shaped by ionotropic gelation process. This process was happened because of the cross-linked between pectin carboxylate groups which had negative load and calcium with the positive ion. Beads obtained in calcium chloride solution was stored for 15 minutes. Then, the beads were separated from the solutions and rinsed with distilled water three times and dried. The drying process then performed in 25°C room temperature for around 2 days.

**Beads coated with pH-sensitive polymer**

Calcium pectinate beads coating was performed to hold tetrandrine release in the upper part of gastrointestinal tract. This experiment was used Eudragit L100-55, Eudragit L100, HPMCP HP-55, or CAP. Calcium pectinate beads containing dry tetrandrine were put into coating solution which had been thickened. After a continuous stirring, beads were separated from the coating solution, dried with a warm air, then separated one by one.

**Beads characterizations**

**Beads shape**

According to the observation, the wet beads were found spherical in a yellowish clear color. After dried on filter paper at room temperature, the beads became brownish yellow, the size was changed and the beads visually still showed spherically. According to the visual observation, Formula 1 had the smallest beads size and the driest textures. Formula 2 had a bigger size than Formula 1 and the beads were found more moisture. Formula 2 beads had a paler color compared with Formula 1 and felt a bit sticky. In Formula 2, beads found in the biggest size, a pale yellow, and had a characteristic like Formula 2 which were found sticky. Formula 1 was determined as the best formula with the best shape and morphology. These results can be seen in Fig. 1.

**Morphology**

According to the SEM results under 1000× magnification (Fig. 2), on Formula 2 and Formula 3 showed that calcium chloride concentrations affected the beads surface characterization, where the higher the calcium chloride concentrations then the beads obtained would be softer and got fewer pores. These results showed that the beads would be more rigid.

**Particle size distribution**

Beads particle size distribution was evaluated using the optical microscope in 10× magnification for Formula 1 and 4× magnification for Formula 2 and 3. Formula 1 which was distributed in 741-810 µm was found in 29%, Formula 2 which was distributed in 1179-1256 µm was found in 37%, and Formula 3 which was distributed in 1179-1256 µm was found in 46.67%.

Coated particle beads size distribution evaluation also performed using an optical microscope with 4× magnification. Formula 1A which was
Experienced evaporation. The acetone usage as the solvent of the coating materials; thus, they had lower water content. This low water content could be caused by the water content of the beads. Moreover, the coated beads were 5-15% calcium chloride had a hygroscopic property so that it high concentrations of calcium chloride in crosslink solutions which concentration of water. The high-water content could be caused by the beads were experiencing size shrinkage after drying process; thus, they lost moisture in the polymer which causing a lower dry beads weight.

Entrapment efficiency
Entrapment efficiency determination was calculated based on the concentration of tetrandrine in the beads. Entrapment efficiency was performed by soaking the beads in phosphate buffer medium pH 6.8 so that the beads would expand and release the drugs. Then, adding HCl to dissolve the tetrandrine. Entrapment efficiency obtained for Formulas 1-3 were 65.67%, 68.03%, and 56.28%, respectively.

Thermal test
The thermal test was performed on tetrandrine, pectin, CaCl₂, calcium pectinate beads, also calcium pectinate containing tetrandrine. The test was performed using differential scanning calorimeter device. Results of tetrandrine thermogram showed an endothermic peak at 219.32°C. Calcium chloride had an endothermic peak at 161.64°C. Pectin had two endothermic peaks which were at 75.70°C and 153.11°C. Empty calcium pectinate beads had two endothermic peaks which were at 88.33°C and 174.56°C. Calcium pectinate beads containing tetrandrine showed an endothermic peak at 179.48°C. These results are illustrated in Fig. 3.

X-ray diffraction
X-ray diffraction test was performed to detect the drug polymorphism after gelation process [14]. The pattern of tetrandrine showed a dominant crystalline phase; this showed by sharp and tall diffractions. The decreased tetrandrine peak intensity can be found in calcium pectinate beads containing tetrandrine; this showed that there was a physical interaction. Tetrandrine was transformed from a crystalline phase to amorphous phase in calcium pectinate beads. These results can be seen in Fig. 4.

Swelling test
Beads ability to swell was observed in phosphate buffer medium pH 6.8 for 3 hrs at room temperature. In phosphate buffer medium pH 6.8 testing, they showed that Formula 1 expanded by 186.35%, Formula 2 expanded by 156.77%, and Formula 3 expanded by 151.17%.

Phosphate buffer pH 6.8 was used as a medium to simulate a colon fluid pH. The aim of swelling ability test was to define the ability of beads to swell when beads had reached the colon. Based on the swelling test result, the increase in calcium chloride concentration reduced the pectin’s ability to swell. By increasing the calcium chloride concentration, reduced the total water that passed through the beads produced particles with denser structures and reducing beads permeability, thus the higher concentration of calcium chloride used then the beads ability to swell was reduced [15].

In-vitro release test
In hydrochloride acid pH 1.2 medium, beads with 10% and 12.5% Eudragit L100-55 formulas showed the cumulative drug release were 21.80% and 10.51%. Eudragit L100-55 in 12.5% concentration could maintain a better drug release. However, beads with Eudragit L100 could maintain a better drug release than Eudragit L100-55. The cumulative drug release in HCl medium for Formula 1C was 3.54% and 1D was 6.33%. While beads coated with phthalate polymer could not resist tetrandrine released better than the methacrylate polymer in acidic medium.

In phosphate buffer pH 7.4, it was expected that the coat still could hold drug release. Absorption showed that the value was starting to increase.
This showed that the drugs slowly had been released from the beads. The cumulative drug release in phosphate buffer pH 7.4 for Formula 1A was found in 37.90% and for Formula 1B was found in 27.97%. Beads with Eudragit L100 coat had a lower drug release compared with Eudragit L100-55, which were 13.58% and 18.29 for Formula 1C and 1D, respectively.

In phosphate buffer pH 6.8, it was expected that the drugs would completely release. The drug release in minute 315 was increased significantly. This was caused by the coating layer which had been eroded and in phosphate buffer pH 6.8, beads could well expand thus it helped the drug release. The cumulative drug release result in Formula 1A was 60.74%, 1B was 54.04%, 1C was 57.87%, and 1D was 66.54%.

After 8 h, all beads coating with phthalate polymer reaching 100% release in the colonic simulated medium, 1E was 113.13 %, and 1F was 109.34 % while 1G was 113.64 % and 1H was 106.95 %. From the test, it can be concluded that beads coating with methacrylate polymer is sufficient to resist tetrandrine released than phthalate polymer.

Based on in-vitro results, beads with 10% Eudragit L100 coat had the lowest drug release cumulative in HCl pH 1.2 and phosphate buffer pH 7.4. Then, when entering phosphate buffer medium pH 6.8, the drug release was significantly increased. Therefore, Formula 1C was chosen to be used in in-vivo targeted test. These results are demonstrated in Fig. 5.

In-vitro drug release was tested in hydrochloride acid pH 1.2 medium as a gastric acid fluid simulation for 2 hr, phosphate buffer pH 7.4 as small intestine fluid simulation for 3 hrs, and phosphate buffer pH 6.8 as colon fluid simulation for 3 hrs. This release test carried out without the presence of enzymes, while in fact, enzymes would trigger more drug release mechanism.

In-vivo targeted test

Beads formula with Eudragit L100 10% coat was chosen as a formulation with the best in-vitro profile which then used in in-vivo drug targeted test using rats. Before the intervention, the rats were fasting for 1 day to clean the gastrointestinal tract from food or feces thus facilitate the observation. According to the time orientation, we chose 2.5 hr as the most suitable dissection time to observe. 2 ½ hrs after beads injection, rats were dissected and observed to define the colon condition. These results were demonstrated in Table 3.

According to the result of control rats, we found no beads in rats gastrointestinal tract, beads without a coat was expected had been degraded by gastric pH before reached the intestine. In rat 1, we found beads in the gastric with the coat still could be found; in rat 2, we found beads in the intestine in 64 cm distance from the end of gastric (antrum) with the thin coat still could be found; then in rat 3, we found beads in the intestine in 90 cm distance from the end of gastric (antrum), beads in this condition found expanded and we could not find any coat left. According to these results, beads reached an average distance of 77 cm from the end of gastric (antrum), which was still in jejunum part [16]. Beads found in rat gastrointestinal tract showed coated beads toleration against pH of the upper gastrointestinal tract. Furthermore, gastrointestinal tract distance from each rat also affect the study results.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal</th>
<th>Observation results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Rat 1</td>
<td>No beads found</td>
</tr>
<tr>
<td></td>
<td>Rat 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat 3</td>
<td></td>
</tr>
<tr>
<td>Calcium pectinate beads coated by Eudragit L-100 10%</td>
<td>Rat 4</td>
<td>Beads found in gastric</td>
</tr>
<tr>
<td></td>
<td>Rat 5</td>
<td>Beads found in intestine in 64 cm distance from the end of gastric</td>
</tr>
<tr>
<td></td>
<td>Rat 6</td>
<td>Beads found in intestine in 90 cm distance from the end of gastric</td>
</tr>
</tbody>
</table>

Table 3: Observation results of in-vivo targeted test
CONCLUSION
Calcium pectinate beads production containing tetrandrine was performed using ionotropic gelation method which produced beads in a spherical shape and brownish yellow color. In shape characterization, surface morphology, size, water content, entrapment efficiency tests, the results differed between the various calcium chloride concentrations used which were 5%, 10%, and 15%. The beads size, water content, and entrapment efficiency were increased following the increased calcium chloride concentration used. Formula 1 used as the best beads characterization. Formula 1 had an average size of 832.23 µm, water content was 28.75%, and the entrapment efficiency was 65.67%. Formula 1 then coated with Eudragit L100-55, Eudragit L100, HPMCP HP-55 or CAP. In addition, in-vitro release study showed that beads which were coated with Eudragit L100 could hold the drug release in the upper gastrointestinal tract better than others. Formula 1C (beads coated with 10% Eudragit L100) was chosen as a formulation with the best in-vitro profile which showed an optimal protection from gastric acid. Moreover, the in-vivo targeted test showed that Formula 1C could deliver the beads to the intestine compared to the control beads.

ACKNOWLEDGMENTS
The authors gratefully thank Dr. Sutriyo and PT. Jebsen Jessen Ingredients, Indonesia for supplying Eudragit L100 and L100-55. The authors also gratefully acknowledge the financial support for this study by Faculty of Pharmacy, Universitas Indonesia Grant Research: Young Lecturer Research (No. 027/JU2.F11.D5/HKP05.00/2016).

REFERENCES