ABSTRACT

Tetrandrine was an alkaloid isolated from *Stephania tetrandra* S. Moore root, studied as an inhibitor working in transforming growth factor (TGF)-β signalling pathway (TGF-inhibitor) [6]. Therefore, it was expected to have potential in intestinal fibrosis treatment. In this study, we developed a colon-targeted preparation, such as tetrandrine beads for the treatment of fibrosis, using ionotropic gelation method, with chitosan as the polymer and TPP as the crosslinker, and also coating the beads using pH sensitive polymer i.e. Eudragit L100-55, Eudragit L100, hydroxypropyl methyl cellulose phthalate (HPMCP) HP-55 or cellulose acetate phthalate (CAP). The final beads then characterized and conducted a test to obtain the drug release profile in vitro and in vivo.

INTRODUCTION

The colon is one of the most important organs in the human body's digestive system. In the treatment of colon-related diseases, it is necessary to deliver a drug compound directly to the active site. This type of delivery systems makes the release and absorption of drugs do not occur in the upper gastrointestinal tract but occurs after the drug reaches the colon [1].

Drug delivery to the colon via oral route can be directly treated a variety of diseases in the colon, such as fibrosis. Tetrandrine is a drug that has anti-fibrosis effects. In this study, chitosan-tripolyphosphate (TPP) beads containing tetrandrine was made and evaluated for in vivo release profile and in vitro targeted test. Tetrandrine was an alkaloid isolated from *Stephania tetrandra* S. Moore root, studied as an inhibitor working in transforming growth factor (TGF)-β signalling pathway (TGF-inhibitor) [6]. Therefore, it was expected to have potential in intestinal fibrosis treatment. In this study, we developed a colon-targeted preparation, such as tetrandrine beads for the treatment of fibrosis, using ionotropic gelation method, with chitosan as the polymer and TPP as the crosslinker, and also coating the beads using pH sensitive polymer i.e. Eudragit L100-55, Eudragit L100, hydroxypropyl methyl cellulose phthalate (HPMCP) HP-55 or cellulose acetate phthalate (CAP). The final beads then characterized and conducted a test to obtain the drug release profile in vitro and in vivo.

MATERIALS AND METHODS

Materials

Tetrandrine (Shaonzi Gyuans Biotech, China), sodium tripolyphosphate (Brataco, Indonesia), chitosan (Biotech Surindo, Indonesia), Eudragit L100-55 (Evonik, Germany; obtained from PT. Jebsen & Jessen Ingredients, Indonesia), Eudragit L100 (Evonik, Germany; obtained from PT. Jebsen & Jessen Ingredients, Indonesia), cellulose acetate phthalate (Eastman, USA; obtain from Eastman Chemical Company, Singapore), hydroxypropyl methyl cellulose phthalate HP-55 (Shin Etsu, Japan; obtained from PT Lawsem Zecha, Indonesia), t alc (Brataco, Indonesia), triethyl citrate (Wefang Limin Chemical, China; obtained from PT. Lawsem Zecha, Indonesia), Tween 80 (Brataco, Indonesia), chloride acid (Merck, Germany), potassium phosphate monobasic (Merck, Germany), sodium hydroxide (Merck, Germany), acetone (Brataco, Indonesia), isoipropanol (Brataco, Indonesia), deionized water (Brataco, Indonesia).

Animals: The *Sprague-Dawley* male rats with a weight of 260-330 g from Badan Penelitian dan Pengembangan Kesehatan (Balitbangkes, Indonesia)

Objective: Drug delivery to the colon via oral route can be directly treated a variety of diseases in the colon, such as fibrosis. Tetrandrine is a drug that has anti-fibrosis effects. In this study, chitosan-tripolyphosphate (TPP) beads containing tetrandrine was made and evaluated for in vitro release profile and in vivo targeted test.

Methods: Chitosan-TPP tetrandrine beads were prepared by an ionic gelation method with variation in sodium tripolyphosphate concentration: 3% (Formula 1), 4% (Formula 2), and 5% (Formula 3). All formulae were characterized for its morphology, particle size, moisture content, process efficiency, entrapment efficiency, thermal character, crystallinity, and swelling. Then, the best formula was coated with HPMCP HP-55, CAP, Eudragit L100-55, or Eudragit L100 prior to drug release profile in vitro and in vivo test.

Results: Beads from all formulae had an average size: 920.50±0.04 µm, 942.21±0.08 µm, and 1085.95±0.03 µm; Water content: 7.28±0.003%, 5.64±0.005%, and 6.84±0.004%; Process efficiency: 29.70%, 28.96%, and 29.70%; Entrapment efficiency: 16.20±0.63%, 17.02±0.37%, and 20.42±0.70% for Formula 1, 2, and 3, respectively. In addition, the results of in vitro cumulative drug release were 67.36%, 76.04%, 83.12%, 83.21%, 40.16%, 37.98%, 45.86%, 41.71% for Formula 3A-3H, respectively.

Conclusion: It can be concluded that Formula 3D (CAP 15%) was chosen as a formulation with the best in vitro profile. Moreover, the in vivo targeted test showed that Formula 3D was able to deliver the beads to the intestine compared to the control beads.

Keywords: Beads, Chitosan, triopolyphosphate, Colon-targeted, Ion gelation, Tetrandrine

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FORMULATION OF CHITOSAN TRIPOLYPHOSPHATE-TETRANDRINE BEADS USING IONIC GELATION METHOD: IN VITRO AND IN VIVO EVALUATION

Original Article
Preparation of chitosan-tripolyphosphate beads

Tetrandrine was dissolved in 0.5 N HCl pH 2, then mixed with 5% (w/v) chitosan solution. A solution of chitosan-tetrandrine then dripped slowly by using 26 G syringe needle into a solution of TPP containing tetrandrine. The X-ray diffraction pattern was recorded was performed on tetrandrine and calcium pectinate beads with a 100 ml/min flow rate. Nitrogen gas was used as purge gas with a 100 ml/min flow rate. Measured from 25-300 °C with the heating rate was 10 °C/min. A total of 5.0 mg sample was put into a crucible 10.0 μl, then heated and dried completely at an elevated temperature until no further weight change was observed. The test was performed using differential scanning calorimetry (DSC; PerkinElmer STA 6000, USA) on tetrandrine, pectin, CaCl₂. The test was performed using differential scanning calorimetry (DSC; PerkinElmer STA 6000, USA) on tetrandrine, pectin, CaCl₂. The sample then observed under vacuum with scanning electron microscope (SEM; LEO 420i, United Kingdom). Beads were coated with gold metal and put in the sample holder. The diameters of 300 beads were measured using an optical microscope. Beads surface shape were observed using an optical microscope. Beads surface shape were observed using an optical microscope. The sample then observed under vacuum with scanning electron microscope (SEM; LEO 420i, United Kingdom).

**Beads coating process with pH-sensitive polymers**

Eudragit L100-55 was mixed with plasticizer and talc thus can be obtained 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 (2.5%, w/w) was used as a plasticizer.

**Table 1: Chitosan-tripolyphosphate beads formula**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Chitosan (% w/v)</th>
<th>Sodium tripolyphosphate (% w/v)</th>
<th>Tetrandrine (% w/w)*</th>
<th>Crosslink time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>3</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>15</td>
</tr>
</tbody>
</table>

*weight ratio between chitosan and tetrandrine (2:1)

**Table 2: Coating formula**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Coating material</th>
<th>Concentration (% w/v)</th>
<th>Plasticizer (%)*</th>
<th>Talc (%)*</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>HPMCP</td>
<td>10</td>
<td>2.5</td>
<td>-</td>
<td>Acetone</td>
</tr>
<tr>
<td>B</td>
<td>HPMCP</td>
<td>12</td>
<td>2.5</td>
<td>-</td>
<td>Acetone</td>
</tr>
<tr>
<td>C</td>
<td>CAP</td>
<td>10</td>
<td>2.5</td>
<td>-</td>
<td>Acetone</td>
</tr>
<tr>
<td>D</td>
<td>CAP</td>
<td>15</td>
<td>2.5</td>
<td>-</td>
<td>Acetone</td>
</tr>
<tr>
<td>E</td>
<td>Eudragit L100-55</td>
<td>10</td>
<td>2.5</td>
<td>5</td>
<td>Acetone-Isopropanol1(1:1)</td>
</tr>
<tr>
<td>F</td>
<td>Eudragit L100-55</td>
<td>12.5</td>
<td>3.125</td>
<td>6.25</td>
<td>Acetone-Isopropanol1(1:1)</td>
</tr>
<tr>
<td>G</td>
<td>Eudragit L100</td>
<td>10</td>
<td>2.5</td>
<td>5</td>
<td>Acetone-Isopropanol1(1:1)</td>
</tr>
<tr>
<td>H</td>
<td>Eudragit L100</td>
<td>12.5</td>
<td>3.125</td>
<td>6.25</td>
<td>Acetone-Isopropanol1(1:1)</td>
</tr>
</tbody>
</table>

*calculated based on coating concentration and solvent

**Beads characterizations**

**Shape**

Beads surface shape were observed using an optical microscope.

**Morphology**

Beads were coated with gold metal and put in the sample holder. The sample then observed under vacuum with scanning electron microscope (SEM; LEO 420i, United Kingdom).

**Particle size distribution**

The diameters of 300 beads were measured using an optical microscope.

**Water content**

Moisture content was determined using moisture balance (AMR 50, United Kingdom). One gram of the beads were weighed. The samples were then placed on the aluminum pan and dried completely at an elevated temperature until no further weight change was observed.

**Thermal test**

The test was performed using differential scanning calorimetry (DSC; PerkinElmer STA 6000, USA) on tetrandrine, pectin, CaCl₂ calcium pectinate beads, also calcium pectinate beads containing tetrandrine. A total of 5.0 mg sample was put into a crucible 10.0 μl, then heated and measured from 25-300 °C with the heating rate was 10 °C/min. Nitrogen gas was used as purge gas with a 100 ml/min flow rate.

**X-ray diffraction**

X-ray diffraction (XRD) test (Philips PW 2213/20, The Netherlands) was performed on tetrandrine and calcium pectinate beads containing tetrandrine. The X-ray diffraction pattern was recorded pH 6.5 under 200 rpm stirring rate for 45 min. Beads obtained in TPP solution was stored for 30 min, then collected and rinsed with deionized water three times. After rinsing, beads were dried at room temperature (25 °C) for 48 h. All beads formulae can be seen in Table 1.

**Entrapment efficiency**

Tetrandrine content test in the beads was performed by weighing carefully ± 50.0 mg beads, then soaked in 10.0 ml phosphate buffer pH 6.8 for 24 h. After 24 h, the solutions were stirred with a magnetic stirrer at 100 rpm and heated in 37 °C until they disintegrated. The disintegrated beads then mixed in phosphate buffer pH 6.8 with HCl 0.1 N addition until obtained 50.0 ml volume, then put into centrifuge tubes and separated from centrifugation device for 10 min at 2500 rpm. After separated, the supernatant was collected then adding HCl 0.1 N until obtaining 100.0 ml. Twenty millilitres solution was pipetted, put in 50.0 ml volumetric flask and adding phosphate buffer pH 6.8 until reaching the absorption limit. Tetrandrine absorption was measured using spectrophotometer UV-Vis (Shimadzu UV-1800, Japan) at 280 nm wavelength. Tetrandrine content was measured by comparing the calibration curve thus the tetrandrine entrapped could be measured.

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

\[
\text{Process efficiency} = \frac{W_m}{W_t} \times 100 \% (1)
\]

\[
W_m = \text{Beads weight obtained (g)}
\]

\[
W_t = \text{Total beads material weight (g)}
\]


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Entrainment efficiency (%) = \frac{\text{Total core measured}}{\text{Total core theoretic}} \times 100\% \tag{2}

Swelling test

Two-point five-gram beads sample from each formula was weighed \(W_1\) then placed on weighing dishes. Twenty-five millilitres phosphate buffer pH 6.8 was added and stayed aside to expand in the room temperature. After 5 min, the sample was collected from the container, carefully dried and the rest of the medium was absorbed by filter paper, then weighed \(W_2\). 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 min. swelling ability was measured using the following formula:

\[
\text{Swelling ability} (%) = \frac{W_2 - W_1}{W_1} \times 100\% \tag{3}
\]

\(W_1\) = Dry sample weight

\(W_2\) = Hydrated sample weight

In vitro release study

The in vitro release study was performed in hydrochloric 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 chloric acid 0.1 N pH 1.2 medium was observed for 2 h, in phosphate buffer pH 7.4 medium was observed in 3 h and the phosphate buffer pH 6.8 medium was observed in 3 h. One hundred milligram beads were weighed and put in the filter bag then put into the dissolution medium. Ten milliters 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-Vis.

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

\[
n = \frac{y_n - a}{x_p} \times \frac{M}{S} + \frac{y_{n-1} - a}{x_p} \times \frac{M}{S} + \ldots + \frac{y_1 - a}{x_p} \times \frac{M}{S} \times 1000 \tag{4}
\]

\(y = \) 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. prior to the experiment procedure, animals have been acclimatized for one week. Rats were placed in the cage with free access to their food and drink. The cage environment was controlled to minimize the humidity and the temperature was maintained around 25 °C. Furthermore, there was a dark and light cycle every 12 h. Each group of rats was placed in a separate cage and maintained in such a way so the rats did not interact with each other. The condition of the rats was monitored every day and the weight of rats was weighed every week. 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 dissection times were 1, 1.5, 2, 2.5, and 3 h [11]. The beads tested on rats were beads with coating formula which provided the best in vitro release profile. Rats were separated into two groups, which were: (1) beads without coating as control and (2) beads with the best coating. The rats were dissected in the time determined before according to 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/UN2.13/ETh/2016.

RESULTS AND DISCUSSION

Chitosan-tripolyphosphate beads

Production was performed with 5% chitosan solution and the concentration of TPP (3%, 4%, and 5%) with tetrandrine and chitosan ratio used was 1:1. Chitosan in 5% concentration was dissolved in deionized water. Then tetrandrine was dissolved in HCl 0.5 N. Chitosan and tetrandrine solutions were mixed and stirred until homogenous using stirrer. When the chitosan and tetrandrine mixtures were shed on TPP, beads would immediately have shaped by ionotropic gelation process. This process was happened because of the cross-linked between chitosan amine groups which had positive load and TPP with the negative ion [12].

Beads coated with pH-sensitive polymer

Chitosan-TPP beads coating was performed to hold tetrandrine release in the upper part of gastrointestinal tract. This experiment was used Eudragit L100-55, Eudragit L100, HPMC HP-55, or CAP. Chitosan-TPP 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 characterization

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 sphericall. These results can be seen in fig. 1.
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 Tween 80 15% (w/v) to dissolve the tetrandrine. Entrapment efficiency obtained for Formula 1, 2, and 3 were 16.20±0.63%, 17.02±0.37%, and 20.42±0.70%, respectively. All characterizations of core beads were described in table 3.

**Thermal test**

The thermal test was performed on tetrandrine, chitosan, sodium tripolyphosphate, chitosan-TPP beads, also chitosan-TPP containing tetrandrine. The test was performed using differential scanning calorimeter device. Results of tetrandrine thermogram showed an endothermic peak at 219.32 °C. Chitosan had one endothermic peak at 259.25 °C and two exothermic peaks at 300.57 °C and 855.69 °C. Sodium tripolyphosphate had four endothermic peaks which were at 58.42 °C, 119.26 °C, 537.74 °C, and 621.97 °C. Chitosan-TPP beads showed an endothermic peak at 192.67 °C, while chitosan-TPP beads containing tetrandrine showed an endothermic peak at 193.55 °C. These results were illustrated in fig. 3.

On DSC thermogram of beads containing tetrandrine we found a different endothermic peak compared with thermogram of tetrandrine and empty beads, and with each beads material. A significant peak change in DSC thermogram confirmed that tetrandrine had experienced chemical interaction and had been entrapped into the beads.

**X-ray diffraction**

X-ray diffraction test was performed to detect the drug polymorphism after gelation process [15]. X-ray diffraction pattern of tetrandrine showed a dominant crystalline phase, this showed by sharp and tall diffractions. The decreased tetrandrine peak intensity can be found in chitosan-TPP beads containing tetrandrine, this showed that there was physical interaction. Tetrandrine was transformed from a crystalline phase to amorphous phase in chitosan-TPP beads (fig. 4).

### Table 3: Characterization of core beads

<table>
<thead>
<tr>
<th>Core beads</th>
<th>Formula</th>
<th>Mean of diameter* (µm)</th>
<th>Water content* (%)</th>
<th>Process efficiency (%)</th>
<th>Mean of entrapment efficiency* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan-TPP beads</td>
<td>1</td>
<td>920±5±0.04</td>
<td>7.28±0.003</td>
<td>29.70</td>
<td>16.20±0.63</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>942±2±0.08</td>
<td>5.64±0.005</td>
<td>28.96</td>
<td>17.02±0.37</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1085±95±0.03</td>
<td>6.84±0.004</td>
<td>29.70</td>
<td>20.42±0.70</td>
</tr>
</tbody>
</table>

*n=3; Data are expressed as mean±SD.
Swelling test

The swelling ratio of beads was observed in HCl pH 1.2 medium for 3 h at room temperature. The results showed that the beads expanded by 192.40%, 195.20%, and 199.20% for Formula 1, 2, and 3, respectively. On the other hand, the swelling ability of chitosan-TPP beads in phosphate buffer pH 6.8 medium was lower, i.e., 128%, 132%, and 136% for Formula 1, 2, and 3, respectively. The swelling ratio was high at pH 1.2 in comparison to 6.8 which is in line with the earlier report by Srinatha et al. [12].

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 the beads had reached the colon. Based on the swelling test result, chitosan-TPP beads was better swell in the acid medium than in pH 6.8 medium. These results might cause by a chitosan-TPP contains-NH$_2$ group which in the acidic conditions can be protonated into NH$_3^+$, Furthermore, because of this condition, made the chitosan became more hydrophilic, thus the chitosan-TPP could swell in the acidic medium [16].

In vitro release test

In HCl pH 1.2 medium, beads with 10% and 15% CAP formulae showed the cumulative drug release were 5.45% and 2.76%, CAP in 10% concentration could maintain a better drug release. However, beads with HPMCP could maintain a better drug release than CAP. The cumulative drug release in HCl medium for formula 3A was 1.72% and 3B was 1.37%. In contrast, beads coated with methacrylate polymer (Eudragit) couldn’t resist tetrandrine released better than the phthalate 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 3A was found in 68.10% and for Formula, 3B was found in 66.47%. On the other hand, beads with CAP coat had lower release value, 43.62% and 36.64% for Formula 3C and 3D. In addition, beads with Eudragit L100 coat had a lower drug release compared with Eudragit L100-55, which was 19% for Formula 3G and 3H.

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 6.8, beads could well expand thus it helped the drug release. In contrary, the drug release on beads with HPMC coat had decreased. It might be all drug already released beforehand. Furthermore, beads with Eudragit—a methacrylate polymer—coat had a lower drug release compared with CAP or HPMC—a phthalate polymer.

After 8 h test, it can be concluded that beads coating either with methacrylate or phthalate polymer was sufficient to resist tetrandrine released in the upper gastrointestinal tract, but phthalate polymer could release more drug at pH 6.8 which was simulated as a colon.
Based on the *in vitro* results, beads with 15% CAP 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 3D was chosen to be used in the *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 h, phosphate buffer pH 7.4 as small intestine fluid simulation for 3 h, and phosphate buffer pH 6.8 as colon fluid simulation for 3 h. 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 CAP 15% 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 one day to clean the gastrointestinal tract from food or feces thus facilitate the observation. According to the time orientation, we chose 2.5 h as the most suitable dissection time for observation. Two and half hours after beads administration, rats were dissected and observed to define the colon condition.

Conforming to the result of control rats, we found no beads in rat gastrointestinal tract, beads without a coat was expected had been degraded by gastric pH before reached the intestine. In rat 1, we found beads at the half of the total intestine length; in rat 2, we found beads at two-third of the total intestine length; then in rat 3, we found beads at three-fourth of the total intestine length. All beads in these conditions found expanded and we could not find any coat left. These results can be seen in fig. 6. Beads found in rat gastrointestinal tract showed coated beads toleration against pH of the upper gastrointestinal tract. Also, gastrointestinal tract distance from each rat also affect the study results.

![Fig. 5: The cumulative drug release profile, a) formula 3A (HPMCP 10%), 3B (HPMCP 12%), 3C (CAP 10%), and 3D (CAP 15%), b) Formula 3E (Eudragit L100-55 10%), 3F (Eudragit L100-55 12.5%), 3G (Eudragit L100 10%), and 3H (Eudragit L100 12.5%). n=3, data are expressed as mean±SD](image)

**Fig. 6: Beads appearance after the *in vivo* test, a) rat 1, b) rat 2, c) rat 3**
CONCLUSION
Together, it can be concluded that the best formulation based on the characterizations was Formula 3 (5% of TPP concentration). Formula 3 had an average size of 1.085±0.03 µm, water content was 6.84%, and the entrapment efficiency was 20.42±0.70%. Formula 3 was then coated with HPMCP HP-55, CAP, Eudragit L100-55 or Eudragit L100. In addition, in vitro release study showed that beads which were coated with CAP 15% could hold the drug release in the upper gastrointestinal tract better than others. Formula 3D (beads coated with 15% CAP) 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 3D could deliver the tetrandrine to the intestine compared to the control beads.

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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/UN2.F11.D5/HKP.05.00/2016).

CONFLICT OF INTERESTS
The authors have no conflict of interest to declare.

REFERENCES

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