ABSTRACT

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 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 EuDrugit 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%, and 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, tripolyphosphate, Tetrandrine, Ion gelation, Polymer, Colon-targeted, Drug delivery
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 (w/v) chitosan solution. A solution of chitosan-tetrandrine then dripped slowly by using 26 G syringe needle into a solution of TPP.

X-ray diffraction (XRD) test (Philips PW 2213/20, The Netherlands) was performed on tetrandrine and calcium pectinate beads containing tetrandrine. A total of 5.0 mg sample was put into a crucible 10.0 μl, then heated and elevated temperature until no further weight change was observed. All beads formulae can be seen in table 1.

Morphology

Beads surface shape were observed using an optical microscope.

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 using diffractometer X-ray radiation with Cu as the anode and graphite monochromatic, operate in 40 kV, 30 mA.

Process efficiency

Process efficiency was defined by comparing total dry beads weight obtained against total material used during the beads production. The recovery value could be obtained with this following formula:

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

Where:
- \( W_m \) = Beads weight obtained (g)
- \( W_t \) = Total beads material weight (g)

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 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 (%) by the following formula:

\[ \text{Entrapment percentage (%) = \frac{W_{m tetrandrine}}{W_{m chitosan}} \times 100\%} \]

Where:
- \( W_{m tetrandrine} \) = Tetrandrine weight obtained (g)
- \( W_{m chitosan} \) = Total chitosan weight (g)

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) solution in acetone were used for coating and triethyl citrate (2.5%, 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 (2.5%, w/w) was used as a plasticizer. Coating formula can be seen in table 2.

Table 1: Chitosan-tripolyphosphate beads formula

| Formula | Chitosan (% w/v) | Sodium tripolyphosphate (% w/v) | Tetrandrine (% w/w) | Crosslink time (min) |
|---------|-----------------|-------------------------------|------------------|----------------------|
| 1       | 5               | 3                             | 2.5              | 15                   |
| 2       | 5               | 4                             | 2.5              | 15                   |
| 3       | 5               | 5                             | 2.5              | 15                   |

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

Table 2: Coating formula

| Formula | Coating material | Concentration, (% w/v) | Plasticizer (%) | Talc (%) | Solvent             |
|---------|-----------------|------------------------|-----------------|----------|---------------------|
| A       | HPMCP           | 10                     | 2.5             | -        | Acetone             |
| B       | HPMCP           | 12                     | 2.5             | -        | Acetone             |
| C       | CAP             | 10                     | 2.5             | -        | Acetone             |
| D       | CAP             | 15                     | 2.5             | -        | Acetone             |
| E       | Eudragit L100-55| 10                     | 2.5             | 5        | Acetone-Isopropanol(1:1) |
| F       | Eudragit L100-55| 12.5                   | 3.125           | 6.25     | Acetone-Isopropanol(1:1) |
| G       | Eudragit L100   | 10                     | 2.5             | 5        | Acetone-Isopropanol(1:1) |
| H       | Eudragit L100   | 12.5                   | 3.125           | 6.25     | Acetone-Isopropanol(1:1) |

*calculated based on coating concentration and solvent

Beads characterization

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.

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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.

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. In the case of coating with HPMCP, a 10% (w/v) and 12% (w/v) solution in acetone were used and triethyl citrate (2.5%, w/w) was used as a plasticizer. Coating formula can be seen in table 2.
Entrapment efficiency (%) = \frac{\text{Total core measured}}{\text{Total core theoretic}} \times 100\% \quad (2)

Swelling test
Two-point five-gram beads sample from each formula was weighed (W1) 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 (W2). 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\% \quad (3)

W1 = Dry sample weight
W2 = Hydrated sample weight

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 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 milligramm beads were weighed and put in the filter bag then put into the dissolution medium. Ten millilitres 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:

\text{n minute} = \frac{(yn - a)xfpaM}{bx1000} + \ldots + \frac{(y15 - a)xfpaS}{bx1000} \quad (4)

y = tetrandrine absorption
yn = tetrandrine absorption in n minute
x = tetrandrine concentration
f = 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 aclimatized 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.F1/ETIK/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 dissoluted in deionized water. 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 spherical. These results can be seen in fig. 1.
Morphology

According to the SEM results under 150x magnification (fig. 2), on Formula 2 and Formula 3 showed that TPP concentrations affected the beads surface characterization, where the higher the TPP concentrations then the beads obtained would be softer and got fewer pores [13]. These results showed that the beads would be more rigid.

Particle size distribution

Beads particle size distribution was evaluated using the optical microscope in 40x magnification. Formula 1 which was distributed in 907-944 μm was found in 33.33%, Formula 2 which was distributed in 900-973 μm was found in 44.33%, and Formula 3 which was distributed in 1003-1028 μm was found in 30%.

Coated particle beads size distribution evaluation also performed using an optical microscope. Formula 3E which was distributed in particle size 1820-1979 μm was found in 27%, Formula 3F which was distributed in particle size 1510-1669 μm was found in 28.33%, Formula 3G which was distributed in particle size 1510-1669 μm was found in 29.66%, Formula 3H which was distributed in particle size 1510-1669 μm was found in 28.66%.

Based on the particle distribution results, the beads produced did not have a uniform particle size distribution. This could be caused by the pressure difference in the shedding process of chitosan and tetrandrine mixtures to the sodium tripolyphosphate solutions. The average beads diameter showed that the larger size was obtained while using a higher concentration of sodium tripolyphosphate used. This proved that the big beads size could be affected by sodium tripolyphosphate concentration. The excess TPP ion may cause all part experience cross-link thus it would create a bigger bead, therefore the higher the sodium tripolyphosphate used then the bigger the beads size obtained [13, 14].

Water content

Results of beads water content from the three formulae showed that the water contained was found between 5.64%-7.28%. Water content results towards the dry beads obtained from Formula 1, 2, and 3 were 7.28±0.003%, 5.64±0.005%, and 6.84±0.004%, respectively.

Process efficiency

Process efficiency was calculated by comparing the total weight of the obtained dry beads to the total of raw materials used during preparation. Beads materials used were chitosan, tetrandrine, and sodium tripolyphosphate. The process efficiency process results of dry beads from Formula 1, 2, and 3 were 29.70%, 28.96%, and 29.70%, respectively.

The process efficiency showed the not too high results 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 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 |
|----------------------------------------|
| **Core beads** | **Formula** | **Mean of diameter** (μm) | **Water content** (%) | **Process efficiency** (%) | **Mean of entrapment efficiency** (%) |
| Chitosan- tripolyphosphate  | 1 | 920.5±0.04 | 7.28±0.003 | 29.70 | 16.20±0.63 |
| beads | 2 | 942.2±0.08 | 5.64±0.005 | 28.96 | 17.02±0.37 |
| | 3 | 1085.95±0.03 | 6.84±0.004 | 29.70 | 20.42±0.70 |

*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$_3^+$ Further, 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 HPMCP 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 HPMCP-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

**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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CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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