Tetrandrine Beads Using Alginate/polyvinyl Alcohol and Alginate-carboxymethyl Cellulose: Not Ideal as Colon-targeted Dosage Form

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Abstract

Objectives: Colon drug delivery system should be able to maintain drug release until the system reaches its target. In this study, beads were selected as drug carrier system to deliver tetrandrine to colon using a combination of two polymers, alginate and polyvinyl alcohol (PVA) and also alginate and carboxymethyl cellulose (CMC). It was hypothesized that alginate/PVA beads and alginate-CMC beads may be able to protect the drug effectively while in the gastrointestinal tract and may deliver the drug at colon under the influence of colonic pH. Materials and Methods: Beads were formulated into six formulae with different concentrations of polymer. Beads were characterized and evaluated for in vitro drug release using dissolution method. Results: Beads’ Formula 3 (alginate and PVA 2:1) and Formula 6 (alginate and CMC 2:1.5) were the best formulae with entrapment efficiency 32.12% and 39.83%, respectively. The drug release test was performed first in hydrochloric acid (HCl) medium for 2 h, then in phosphate-buffered pH 7.4 + 2% Tween 80 for 3 h, and finally in phosphate-buffered pH 6.8 + 2% Tween 80 medium for 3 h, successively. The results of drug release in HCl pH 1.2 were 84.13%, 73.12%, 66.57%, 85.59%, 78.26%, and 69.56%, for Formula 1, 2, 3, 4, 5, and 6, respectively. Conclusion: All type of beads showed the high release of tetrandrine in HCl; hence, it is not ideal yet as a colon-targeted dosage form.

Keywords: Alginate, beads, carboxymethyl cellulose, ionic gelation, polyvinyl alcohol, tetrandrine

INTRODUCTION

The colon-specific drug delivery system should prevent the release of drug in the upper part of gastrointestinal tract. This delivery system should be able to protect drug until it reaches the target. The release and absorption of the drug should not occur in the stomach as well as the small intestine but only release and absorbed once the system reaches the colon. The colon-specific drug delivery system is considered to treat a variety of colonic diseases such as ulcerative colitis, Crohn’s disease, amebiasis, and colon cancer.

Intestinal fibrosis is one of the colonic diseases. Intestinal fibrosis is caused by an excess deposition of the extracellular matrix resulting from chronic inflammation and impaired wound healing in the intestine. One of the active substances that can be used to treat fibrosis is tetrandrine. Tetrandrine is one of the traditional Chinese herbal that has pharmacological activities such as anti-inflammatory, antiallergic, antioxidant, and antifibrosis. Treatment of inflammation that occurs in the intestinal mucosal layer will be more effective if the drug can be released on the site of that inflammation. Therefore, a system that can deliver tetrandrine to the colon is required.

Beads are chosen as a carrier to deliver tetrandrine to colon because beads can control the release of the drug. Beads are made using ionic gelation method because of the simple process. The ionic gelation is the ability of polyelectrolyte polymer to cross-link by counterions. The properties of sodium alginate are biocompatible, biodegradable, nontoxic, mucoadhesive, and pH-sensitive polymers. Sodium alginate is cross-linked by calcium to obtain a calcium alginate. Calcium alginate beads have a pH-dependent release profile.
which is stable in acidic media and swelling at pH neutral.[13] However, there are some deficiencies of calcium alginate beads such as low entrapment efficiency and rapid drug release at pH neutral.[13] Hence, this research used a combination of two polymers to overcome those deficiencies.

Carboxymethyl cellulose (CMC) is an anionic and pH-dependent polymer. These properties could retain the release of the drug in the stomach and facilitate rapid release of the drug in the colon. The calcium alginate-CMC beads may be capable of protecting drug in the upper site gastrointestinal tract and deliver the drug to the colon.[13] Polyvinyl alcohol (PVA) is a water-soluble nonionic linear polymer widely used in the biomedical and pharmaceutical because of noncarcinogenic and biocompatible properties. PVA can form a compact network from macromolecule in the blend beads due to the formation of semi-interpenetrating polymer network (semi-IPN). Therefore, penetration of medium through sodium alginate/PVA beads and then diffusion of the drug to the external medium is more difficult compared to the sodium alginate beads.[13] The addition of PVA can increase the entrapment efficiency and capable of decreasing degradation of beads at a pH neutral.[14]

**Materials and Methods**

Tetrandrine (Shaanxi Ciyuan Biotech, China), tetrandrine standard (Sigma-Aldrich, Singapore), sodium alginate (Shandong Jiejing Group Co., China), calcium chloride (Merck, Germany), sodium CMC (Koriko, Indonesia), PVA (Koriko, Indonesia), chloride acid (Brataco, Indonesia), potassium phosphate monobasic (Merck, Germany), sodium hydroxide (Brataco, Indonesia), ethanol (Brataco, Indonesia), Tween 80 (Brataco, Indonesia), and deionized water (Brataco, Indonesia) were used in this study.

**Preparation of calcium alginate/polyvinyl alcohol beads and calcium alginate-carboxymethyl cellulose beads**

PVA solution was prepared by dissolving PVA powder in deionized water at 96°C, and the obtained homogeneous solution was slowly cooled to room temperature. Alginate solution was added to PVA solution. Tetrandrine was dissolved in hydrochloric acid (HCl) 0.1 N, and then, tetrandrine solution was added to mixture polymer solution and stirred until obtained homogeneous solution. After that, the solution was extruded in the form of droplets into 2% calcium chloride solution using syringe needle 26 G under stirring at 200 rpm for 15 min. Then, beads were washed with deionized water and dried at room temperature. Preparation of calcium alginate-CMC beads had the same way with the preparation of calcium alginate/PVA beads [Table 1].

**Morphological characterization**

The shape, color, odor, and surface texture of the beads were observed visually.

**Scanning electron microscope**

The shape and morphology beads were observed using scanning electron microscope (SEM) (Hitachi SU3500, Japan). Beads were placed in the sample holder. The sample then observed under vacuum with SEM.[15]

**Particle size distribution**

The diameters of 300 beads were measured using calipers.

**Determination of moisture content**

Moisture content was measured using moisture balance (Adam, USA). About 1 g of beads were placed in an aluminum pan. Moisture content percentage was determined until no further weight change was observed.

**Process efficiency**

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

\[
\text{Process Efficiency} (\%) = \frac{\text{Weight of the dried beads (gram)}}{\text{Total weight of material used (gram)}} \times 100\%
\]

**Entrapment efficiency and drug content determination**

Entrapment efficiency was measured after extracted tetrandrine from beads. About 30 mg beads were weighed then soaked in 10 ml phosphate-buffered pH 6.8 for 24 h and stirred using magnetic stirrer at 100 rpm and heated in 37°C until the beads disintegrated then adding HCl 0.1 N until 50.0 ml and centrifuged at 2500 rpm for 15 min. After separated, the supernatant was collected and adding HCl 0.1 N to 50.0 ml. Then, the solution was measured using ultraviolet (UV)-visible spectrophotometer (Shimadzu UV-1800, Japan) at 280 nm.

**Table 1: Composition of the calcium alginate/polyvinyl alcohol beads and calcium alginate-carboxymethyl cellulose beads containing tetrandrine**

<table>
<thead>
<tr>
<th>Alginate (%, w/v)</th>
<th>Calcium chloride (% , w/v)</th>
<th>PVA (%, w/v)</th>
<th>CMC (%, w/v)</th>
<th>Tetrandrine (% , w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F1</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>2</td>
<td>2</td>
<td>0.75</td>
<td>-</td>
</tr>
<tr>
<td>F3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
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<tr>
<td>F4</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>0.5</td>
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<tr>
<td>F5</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>F6</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>1.5</td>
</tr>
</tbody>
</table>

CMC: Carboxymethyl cellulose, PVA: Polyvinyl alcohol
Determination of drug loading was calculated by comparing absorbance of the sample on curve calibration.[16]

Entrapment efficiency (%) =

\[
\text{Practical drug loading (mg)} \times 100%
\]

Amount of beads (mg)

Drug content (%) =

\[
\text{Practical drug loading (mg)} \times 100%
\]

Amount of beads (mg)

**Differential scanning calorimetry**

The analysis was performed using differential scanning calorimeter (DSC8000 Perkin Elmer, USA). About 5 mg sample was heated from 30°C to 350°C at a heating rate of 10°C/min.[19]

**X-ray diffraction**

The analysis was performed using diffractometer with Cu anode, running at 40 kV and 40 mA, scanning from 3° to 60° at 15°/min.[17]

**Swelling index analysis**

Swelling of the beads was carried out in phosphate-buffered solution pH 6.8 as simulated intestinal fluid. About 1 g beads were placed at 37°C in 25 ml phosphate-buffered solution. At regular intervals (5, 10, 15, 30, 45, and 60 min), the beads were reweighed. The weight change of the beads was determined as follows:[18]

Percentage of swelling index (%) =

\[
\frac{W_2 - W_1}{W_1} \times 100%
\]

W<sub>1</sub> = Weights of dried beads
W<sub>2</sub> = Weights of swollen beads

**Fourier transform-infrared spectroscopy analysis**

Fourier transform-infrared (FTIR) spectroscopy study was carried out to check the possible interaction between the components in prepared formulations.[19] FTIR spectra were taken in the wavelength region 400–4000/cm using KBr pellet (FTIR 8400S Shimadzu, Japan).[20]

**In vitro drug release studies**

About 100 mg beads were weighed and put in the filter bag then put into the dissolution medium. The beads were initially incubated in 200 ml 0.1 N HCl pH 1.2 (simulated gastric fluid) for 2 h, then incubated in 200 ml of phosphate-buffered solution pH 7.4 + Tween 80 2% (simulated small intestinal fluid) for 3 h, and finally transferred into 200 ml of phosphate-buffered solution pH 6.8 + Tween 80 2% (simulated colonic fluid) for 3 h. Ten milliliter solution was withdrawn at specific time intervals, and drug content was determined by UV-visible spectrophotometer at 280 nm. An equal volume of medium was replaced with release medium to maintain a constant volume. Measurement of the substance contained in the sample at n minute was measured using the following formula:

\[
n\text{ minute (mg)} = \frac{(y_n - a) \times \text{fp} \times M}{b \times 1000} + \ldots + \frac{(y_n - a) \times \text{fp} \times S}{b \times 1000}
\]

yn = tetrandrine absorption in n minute
fp = dissolution factor
M = volume of release medium
S = sampling volume
a = intercept coefficient
b = slope

**RESULTS**

**Morphological characterization**

Wet beads of all formulae were white, spherical, and odorless and had a smooth surface texture while dried beads of all formulae were less spherical, brownish yellow, and odorless and had a rough surface texture. These results can be seen in Figure 1.

**Scanning electron microscope**

Beads which were scanned with ×500 magnification showed a rough surface, crevices, and pores [Figure 2].

<table>
<thead>
<tr>
<th>Table 2: Characteristic results of average diameter, moisture content, process efficiency, entrapment efficiency, and drug content</th>
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</thead>
<tbody>
<tr>
<td><strong>Average diameter (μm)</strong></td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>F1</td>
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<td>F6</td>
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SD: Standard deviation
Particle size distribution
Particle size distribution results [Table 2] showed that with an increase in the polymer concentration, both PVA and CMC, the viscosity of the solution increases which contributed toward higher retention of liquid volume at the tip of the nozzles and thus increased bead size.[31]

Determination of moisture content
Each formula had a low water content because the water from the uncoated beads seeped out of the beads immediately during the drying process.[21] These results can be seen in Table 2.

Process efficiency
The results of process efficiency were 24.48%, 27.67%, 26.57%, 26.04%, 38.50%, 42.62%, and 39.03% for control beads, F1, F2, F3, F4, F5, and F6, respectively.

Entrapment efficiency and drug content determination
Table 2 showed that the entrapment efficiency is affected by the solubility of the drug inside the matrix, formed pores, and polymer concentration while tetrandrine is soluble in the matrix.[22] Furthermore, polymer concentration, drug concentration, and cross-linking agent used were not optimal and caused loss of drug during preparation.[23] The entrapment efficiency increased with increasing the concentration of PVA and CMC resulting in the formation of larger beads entrapping the greater amount of the drug.[24] According to the SEM results, the higher concentration of polymers (PVA or CMC), produced beads with less pores and crevices. The addition of PVA and CMC will increase the density of beads.

Differential scanning calorimetry
The DSC thermograms showed that alginate had an endothermic peak at 80.22°C and an exothermic peak at 249.08°C. CMC had an endothermic peak at 87.05°C and an exothermic peak at 290.68°C. Calcium chloride had two endothermic peaks at 48.92°C and 175.60°C. Tetrandrine had an endothermic peak at 223.84°C. Calcium alginate-CMC beads had two endothermic peaks at 80.22°C and 214.39°C. Calcium alginate-CMC beads containing tetrandrine had an endothermic peak at 81.08°C and an exothermic peak at 281.88°C. PVA had an endothermic peak at 191.90°C. Calcium alginate/PVA beads had four melting points at 76.09°C, 136.65°C, 189.25°C, and 192.87°C and also an exothermic peak at 276.36°C. Calcium alginate/PVA beads containing tetrandrine had two melting points at 76.09°C and 136.61°C [Figure 3].

X-ray diffraction
The analysis of tetrandrine showed sharp and high diffraction peaks that indicated crystalline phase.[25] Diffractogram of alginate-CMC and alginate/PVA beads showed a decrease in intensity of tetrandrine peak, even no sharp peak was observed, suggesting that a decrease in the drug crystallinity degree which may be due to tetrandrine was dispersed inside the polymeric matrix of the beads.[25] This also showed that tetrandrine transformed from crystalline to its amorphous phase.[3] [Figure 4].

Swelling index
The swelling index percentage of control beads, F1, F2, F3, F4, F5, and F6 reached 1545.81%, 1428.14%, 1257.36%, 1129.76%, 1280.10%, 1104.90%, and 1197.25% in 1 h, respectively. It was observed that the swelling index decreased along with the increase of PVA concentration.

Fourier transform–infrared spectroscopy analysis
FTIR spectra of alginate showed a broad peak at 3124.79/cm that indicated hydroxyl group (−OH). Peaks at 1606.76/cm and 1413.87/cm showed the presence of carboxylate group.[26] PVA spectra showed a peak at 3215/cm (−OH) and 1097/cm (C-O). CMC presented a broad peak at 3234.73/cm (−OH). The peak at 1649.19/cm and 1421.58/cm was attributed to the carboxylate group. Broad peaks between 1149.61/cm and 1003.02/cm represented glucose ring.[27] Tetrandrine spectra showed peaks at 1022.31–1066.67/cm and 1224.84–1269.20/cm.

Figure 1: Morphological characteristics of (a) wet beads of calcium alginate/polyvinyl alcohol, (b) dried beads of calcium alginate/polyvinyl alcohol, (c) wet beads of calcium alginate-carboxymethyl cellulose, and (d) dried beads of calcium alginate-carboxymethyl cellulose

Figure 2: Scanning electron micrograph (under ×500) of (a) control beads, (b) F1, (c) F2, (d) F3, (e) F4, (f) F5, and (g) F6
indicating ether group. The peak at 630.74/cm was attributed to an aromatic ring. These results are demonstrated in Figure 5.

**In vitro release study**

Colon-specific drug delivery system should release a minimum amount of drug in the stomach and small intestine. In this study [Figure 6], the beads released a high number of drugs in HCl medium with pH of 1.2 (stomach environment), this was due to the weak alginate/PVA network beads formed and the unstable bond between calcium alginate and CMC. When PVA was added to calcium alginate matrix, semi-IPN will be formed. Addition of PVA in the bead polymeric solution still could not produce bead network that could avoid medium penetration which can be seen from SEM images that all formulae had pores and crevices. The matrix must be strengthened by increasing the gelation profile of PVA, using freeze-thawing, or chemically by using cross-linking agent, i.e., glutaraldehyde.

**Discussions**

Our recent study reported that the size of beads was shrunk during drying process causing crevices in the beads surface. CMC has a higher viscosity than PVA that caused the beads had a higher average diameter. Each formula was prepared by extruding active ingredient and polymer mixture solution using a syringe (26-G), but the beads showed heterogeneous particle size distribution. The preparation process was done manually that pressure differences during extrusion cannot be avoided.

CMC is hygroscopic and absorbs significant amounts of water. This was the reason calcium alginate-CMC bead water content was higher than control beads and calcium alginate/PVA beads. Low efficiencies were observed because the preparation was done manually that there were residues left inside the syringe or container.

In addition, calcium alginate-CMC beads had a higher melting point than alginate, CMC, and calcium chloride. The melting point shift suggested that there was an interaction, where the cross-linking reaction between bead-forming components occurred. The endothermic peaks at 189.25°C and 192.87°C on calcium alginate/PVA beads signified cross-linking between alginate and calcium ion. The exothermic shift of alginate from 248.06°C to 276.36°C on calcium alginate/PVA beads showed an interaction between PVA and alginate. The loss of tetrandrine’s endothermic peak in calcium alginate/PVA beads and calcium alginate/CMC beads, both containing tetrandrine, showed that tetrandrine was distributed molecularly throughout the beads.

Next, the addition of PVA tended to increase the density of the beads that it was harder for water to penetrate inside the beads. Furthermore, the concentration of alginate was decreased that caused the swelling ability to reduce as well. However, the swelling index of alginate-CMC beads was lower than alginate/PVA beads which were due to the increase in CMC concentration that the beads charge density tended to increase. Swelling of the beads occurred because alginate and CMC are pH-dependent anionic biopolymers. Negative charge from carboxylate groups allowed the polymer to shrink in acidic pH and swell in a neutral pH. At an acidic pH, carboxylate groups are not dissociated, and therefore, no charge is developed in the polymeric matrix. When exposed to a neutral medium, carboxylate groups are converted to a negatively charged carboxylate ions that cause repulsion in the polymer chains. This causes the polymeric matrix to swell.

Furthermore, cross-linking of alginate and calcium chloride caused a shift of carboxylate ion wavenumber to a lower wavenumber that indicated a bond between COO⁻ groups and Ca²⁺ ions. Carboxylate groups in calcium alginate-CMC beads also bonded with calcium ions from calcium chloride. Calcium alginate-CMC and alginate/PVA beads loaded with tetrandrine spectra showed no difference compared to unloaded bead spectra because there was no bond between polymer and tetrandrine.

Alginate/PVA and alginate-CMC beads produced porous beads with crevices. This made medium penetration possible through the crevices at the surface. Tetrandrine was soluble in an acidic environment when HCl medium pH 1.2
penetrated inside the beads; the medium would dissolve most of the tetrandrine contained. The drug release profile showed that increasing PVA or CMC concentration would decrease the release of tetrandrine because the thickness of beads layer acted as a barrier that can decrease drug diffusion from swelling beads. Moreover, when alginate is combined with other polymers, PVA and CMC strengthen the beads compared to the beads which made of the only alginate.

**Conclusion**

All type of beads showed the high release of tetrandrine in HCl pH 1.2. Therefore, it can be concluded that calcium alginate/ PVA and calcium alginate-CMC beads containing tetrandrine are not ideal as colon-targeted dosage form.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

5. Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel
Iswandana, et al.: Tetrandrine beads – Not ideal as colon-targeted dosage form