Release Profile of Betanin from Chitosan Microparticle Containing Beetroot (*Beta vulgaris* Linn) Extract

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Abstract. Beetroot (*Beta vulgaris* L.) was formulated into Microparticles (MP) to maintain its antioxidant stability and control the release of active substances for longer use. Chitosan was used as a matrix to encapsulate beetroot extract. This study aims to determine the effect of chitosan concentration on drug loading, encapsulation efficiency, and release profile of betanin from MP containing beetroot extract. Three formulas of microparticles were prepared using the ionic gelation method with concentrations of chitosan 0.5% w/v, 1% w/v, and 2% w/v. Encapsulation efficiency was analyzed using the direct method, and the release profile of the active substance was evaluated using the dissolution method utilizing distilled water as a medium. The results showed that increasing the chitosan concentration from 1 to 2% could reduce the encapsulation efficiency. The release profile of beetroot extract microparticles from chitosan microparticles followed the Higuchi model. The release rate constants of beetroot extract microparticles revealed that using a 2% w/v concentration of chitosan could reduce betanin release rate from beetroot extract microparticles compared to chitosan matrix concentrations of 0.5% w/v and 1% w/v.

Keywords: beetroot · chitosan · drug release microparticle

1 Introduction

Beetroot (*Beta vulgaris* L.) has a potent antiradical and antioxidant activity from betalain, consisting of betacyanin (red-purple) and betaxanthin (yellow) [1]. These compounds have water-soluble properties and are easily degraded under the influence of pH, temperature, and light [2]. However, the antioxidant activity in beetroot has a limitation on stability. Therefore, it is necessary to design a delivery system for the active substance that can effectively protect the stability of beetroot in the long term, such as microencapsulation.

Encapsulation technique using Microparticle (MP) can be used to protect the core substance from damage during the manufacturing and storage process by coating its core material [3]. Microparticle (MP) is also a delivery system for active substances that can

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control their release, improving the stability of active substances. This microencapsulation technique can also be used to control the release of active compounds, provide better stability active compounds, protect materials that are sensitive to the environment, and protect against unwanted effects due to the influence of light, humidity, and oxygen [4].

Moreover, microparticles (MP) are known to protect the active ingredients from factors that cause damage, such as changes in temperature, humidity, oxygen and microorganisms[5, 6]. In the manufacture of microparticles, it is necessary to have a matrix for the active ingredient encapsulation process. Here, chitosan is a polymer that can be used for the encapsulation process. Chitosan has amino and reactive hydroxy groups that have the potential to be further modified [7]. In addition, chitosan has good adhesion properties, minimal toxicity, mechanical strength, hydrophilicity, and stability [8].

As a microparticle polymer, chitosan has a positively charged primary amine group that can be cross-linked to form a gel through ionic interactions with polyanionic compounds, including tripolyphosphate (TPP). Malangngi *et al.* [9] synthesized and modified a thin layer of chitosan-tripolyphosphate as a drug coating material to control drug release. The cross-linked chitosan and tripolyphosphate formed were more stable to swelling. Nussinovitch [10] also stated that chitosan-tripolyphosphate microparticles have better mechanical strength, and the force required to break microparticles is about 10 times that of chitosan-sulfate or chitosan-citrate microparticles [10].

In the microparticle preparation process, the matrix's concentration can affect the microparticles' characteristics, including the encapsulation efficiency (EE) of the active ingredient on the matrix. To obtain good physical characteristics and absorption efficiency of active ingredients, it is possible to evaluate the appropriate matrix concentration in the manufacture of microparticles. The chitosan concentration as a microparticle matrix can affect the release profile of the active ingredient from the microparticle preparation. Research conducted by Sukmawati *et al.* [11] showed that the release of doxorubicin and PGV-1 was influenced by the concentration of the chitosan matrix used in the manufacture. The other research revealed that using sodium alginate as a matrix in mefenamic acid microparticles reduced the release rate of active substances [12]. In this research, the release profile of active antioxidant ingredients of beetroot from microparticle preparation was investigated. The various concentration of chitosan as a matrix in MP were used to maintain the release of betanin for a long period and maintained for a prolonged period.

2 Materials and Method

2.1 Material

The materials used in this study were beetroot (*Beta vulgaris*, Linn) obtained from Cepogo, Boyolali, Central Java, Indonesia, tween 80, chitosan (MW 100–200 kDa, CV Chi Multiguna, Indonesia), acetic acid glacial, sodium tripolyphosphate (Na TPP), citric acid (Merck), distilled water, betanin (Aldrich). Unless otherwise stated, all materials were in pharmaceutical grade and provided by Bratachem, Indonesia.

2.2 Preparation of Beetroot Dry Extract

A peeled beetroot (200 g) was cut into small pieces and mashed with 200 mL of 1% citric acid until smooth using a blender. The mashed beetroot was then filtered using a clean cloth to obtain the juice. The beetroot juice was then dried using freeze-dry until the dry mass was obtained. The yield of dry beetroot powder using this process was 3.23%.

2.3 Preparation of Microparticle

The microparticle (MP) containing beetroot extract was made using three various concentrations of chitosan. A 1.25 g of chitosan was dissolved in 250 mL, 125 mL, and 62.5 mL of 1% acetic acid to give chitosan concentration 0.5%, 1%, and 2% *w/v*, respectively. The solution was stirred using a magnetic stirrer for two hours at a speed of 700 rpm to produce a clear chitosan solution. A 0.625 g of beetroot powder as an active substance for each formula was dissolved in 5 mL of distilled water and added to the chitosan solution. The mixture was stirred for ten minutes at a speed of 350 rpm. Tween 80 was then added to the mixture to give a concentration of 0.2% v/v to stabilize particles in the solution and prevent clumping between particles [13]. A 10 mL sodium tripolyphosphate (Na TPP) 1% *w/v* as a cross-linking agent was added dropwise into each formula using a sprayer. The solution was continuously stirred at 350 rpm for 24 h to perfect the cross-linking process. The solution was then centrifuged at 3000 rpm for 15 min to precipitate the particle. The particle obtained was then washed three times using 3 mL of distilled water. The wet particle was then dried utilizing a freeze dryer for three days and stored at 4 °C in a container covered with aluminum foil.

2.4 Evaluation of Drug Loading (DL) and Encapsulation Efficiency (EE)

Beetroot extract's Drug Loading (DL) and Encapsulation Efficiency (EE) were evaluated using the direct method and calculated using betanin as a standard. A 50 mg of microparticle was dissolved in 1 mL of 1% glacial acetic acid to dissolve chitosan matrix and then mixed with 4 mL of distilled water in the ratio of 1:4. Then, the solution was centrifuged at 3000 rpm for five minutes to precipitate the chitosan debris. The absorbance of clear solution was measured with a UV-Vis spectrophotometer (Genesys 10S) at 532 nm. The amount of betanin in encapsulated beetroot extract was determined using betanin calibration curve $Y = 0.0002\ x - 0.0325$. DL and EE of betanin in MP were calculated using Eq. (1) and Eq. (2).

$$\text{DL (\% w/w)} = \frac{\text{amount of betanin in MP}}{\text{amount of microparticles}} \times 100\% \tag{1}$$

$$\text{EE (\%)} = \frac{\text{amount of betanin in MP}}{\text{amount of beetroot added}} \times 100\% \tag{2}$$

2.5 Evaluation of Betanin Release Profile from Microparticles

The release of betanin as the active substance from chitosan microparticles was carried out using the dissolution method. A 250 mg of MP were dispersed in a 20 ml tube containing 5 mL of distilled water as a dissolution medium. The tube was placed in a shaking thermostatic water bath (Julabo SW 22) at 37°C ± 0.5 and shaking speed of 150 rpm. The betanin released from the MP was evaluated at certain time intervals from 2 to 180 min by taking out 5 mL of the dissolution medium. Before sampling, the tube was centrifuged for two minutes at a speed of 3000 rpm. Therefore, the MP could settle in the bottom of the tube. The dissolution medium was replaced with the same volume in each sampling time. The clear sample solution was then measured using a UV-V spectrophotometer (Genesys 10s) at 532 nm to evaluate the amount of betanin released from the MP using betanin calibration curve Y = 0,0002 x – 0,0325. The amount of betanin released from MP was then plotted into zero-order (Eq. (3)), first-order (Eq. (4)), second-order (Eq. (5)), and Higuchi model (Eq. (6)) to determine the release kinetics of active substance from MP.

$$C = K_{0.}T \tag{3}$$

$$\text{Log } C = \text{Log } C_{0.} - \frac{K.t}{2.303} \tag{4}$$

$$\frac{1}{C} = \frac{1}{C_0} + K.t \tag{5}$$

$$Qt = K_H \,.\, t^{1/2} \tag{6}$$

Whereas C is the concentration of betanin releases, C_0 is the initial concentration of betanin, K is the release constant, Q_t is the amount of betanin released, K_H is Higuchi released constant, and t is time (minutes).

3 Results and Discussion

The drying process of beetroot extract was carried out using freeze-drying as the active substance is susceptible to high temperatures. Betanin compounds in beetroot are affected by temperature, pH, light, and oxygen; therefore, citric acid was used to maintain the stability of betanin as an antioxidant. According to Kendall and Sofos [14], citric acid was used to maintain pH stability and the natural color of a product due to the ability of citric acid to decrease the pH of substances so that it can reduce the occurrence of enzymatic browning and maintain the stability of color in the dry product.

Microparticle was made using the ionic gelation method. This method involves a cross-linking process between the polyelectrolytes in the presence of their multivalent ion pairs. The reaction mechanism involved the process of dissolving chitosan in 1% glacial acetic acid solution through a protonation reaction. The amine group accepts H^+ released by acetic acid to become positively charged ($-NH_3^+$). The formation of these ions causes the chitosan to be dissolved. The cross-linking process occurs when

Table 1. Drug Loading and Encapsulation Efficiency of Chitosan Microparticle Containing Beetroot Extract in Various Chitosan Concentration

Chitosan Concentration (%)	Drug Loading (%*w/w*)*	Encapsulation Efficiency (%)[a]
0.5%	43.03 ± 2.091	86.05 ± 4.181
1%	46.07 ± 1.392	92.15 ± 2.785
2%	26.41 ± 1.259	52.40 ± 2.498

[a] calculated as betanin. Values represent mean ± SD (n = 3).

the positively charged amine group (cation) cross-linked with the negative group of the tripolyphosphate polyanion to form a complex reaction [13].

Evaluation of DL and EE was carried out to evaluate the method's effectiveness in encapsulating an active substance. DL showed how much beetroot extract (calculated as betanin) was entrapped inside the chitosan microparticles, while EE was used to describe the beetroot's effectiveness entrapment process of beetroot into microparticles. The DL and EE of beetroot in chitosan MP with 0.5, 1, and 2% *w/v* chitosan concentration can be seen in Table 1.

The highest drug loading results were obtained at 1% chitosan concentration, while the lowest was 2%. According to Joshi *et al*. [15], increasing polymer concentration will enhance the polymer's cross-linking ability to bind the active substance, resulting in higher drug loading and encapsulation efficiency. However, the DL obtained in this study is not in accordance with Joshi's statement because the microparticles with the largest chitosan matrix concentration of 2% obtained the lowest DL value. According to Ko *et al.* (2002), the high concentration of chitosan used in the formulation would produce the high viscosity of the chitosan solution. As a result, it formed strong and thick microparticle walls when interacting with Na TPP so that the swelling ability of chitosan decreased. In addition, the high viscosity of chitosan increased the density of the matrix; thereby, it reduced the ability of the chitosan to swell and absorb the active ingredient [16]. Consequently, only a small amount of beetroot extract adsorbed within the microparticle.

The t-test on the encapsulation efficiency data revealed that the chitosan matrix concentration from 1% to 2% had a significant decrease in the EE of chitosan MP ($p < 0.05$), while the increasing chitosan matrix concentration from 0.5% to 1% showed no significant difference in EE of chitosan MP ($p > 0.05$).

The release of the active substance from the chitosan microparticles is influenced by the concentration of the matrix and the type of polymer used in the formulation. Moreover, the release of the active substance from the matrix is also influenced by the solubility of the active substance in the dissolution medium [17] and polymer viscosity, polymer mixture, and particle size [18]. The result revealed that the cumulative percentages of betanin released in 180 min from MP chitosan in concentrations 0.5%, 1%, and 2% were 77.6% ± 4.68, 78.6% ± 4.78, and 66.2% ± 3.14, respectively (Fig. 1). The highest cumulative percentage of betanin released was obtained at 1% chitosan concentration, and the lowest was obtained at 2% chitosan concentration. As the polymer

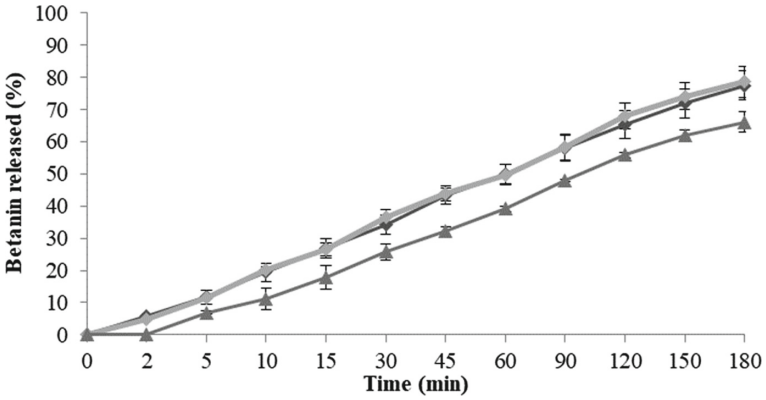

Fig. 1. Cummulative percentage of betanin relesed from chitosan microparticle with concentration 0.5% (◆), 1% (◆), and 2% (▲). Bar represents SD (n = 3).

concentration increased, it would reduce the diffusion rate, so the active substance had a slower release profile [19].

Additionally, the MP at a concentration of 2% chitosan had the lowest encapsulation efficiency. As a result, only a small amount of betanin was adsorbed in the matrix, influencing the percentage of betanin released from the microparticle.

The kinetics of betanin release from beetroot extract microparticles was determined by plotting the amount of betanin released to the zero-order, first-order, second-order, and Higuchi model equations. The release kinetics of the active substance can be determined from the R^2 value of linear regression, which is close to 1. The R^2 value of each released model can be seen in Table 2. The R^2 value close to 1 was found in the Higuchi model; thus, the release kinetics of betanin from chitosan MP followed the Higuchi model. The Higuchi model indicates that the active substance's release mechanism is controlled by diffusion [20]. All the MP in various chitosan concentrations had a Higuchi release model, indicating that the polymer concentration did not affect the release model of betanin from chitosan MP ($p > 0.05$). In general, the release of the active substance from a matrix with low solubility in water will follow the Higuchi model [21]. The release of the active substance from the polymer matrix can occur by diffusion, polymer degradation, or both. Diffusion release occurs when the active substance flows through the pores in the matrix or the spaces between polymer chains [12].

The release constant of betanin from chitosan MP was then determined using the Higuchi release model. The greater the Higuchi constant, the greater the release rate and vice versa. The Higuchi constant for release of betanin from MP showed that the slowest release rate appeared at chitosan matrix 2% w/v (Table 3).

According to Saharan *et al.* (2015), the release of glipizide from microparticles with poly-lactic acid (PLA) matrix decreased by increasing polymer concentration used in the manufacture of glipizide microparticles [22]. In this research, the higher concentration of chitosan during the preparation of microparticles would increase the viscosity of the environment; therefore, it induced the particle compaction process to be faster. Consequently, the process of releasing the active substance would be delayed.

Table 2. The R2 Value of Betanin Release Profile by Plotting Using Zero-, First-, Second-Order, and Higuchi model

Chitosan Concentration (%)	R^2 value[a]			
	Zero-order	First-order	Second-order	Higuchi
0.5	0.9094 ± 0.0094	0.6640 ± 0.0229	0.3637 ± 0.0332	0.9903 ± 0.0028
1	0.9072 ± 0.0071	0.6390 ± 0.0144	0.3206 ± 0.0154	0.9899 ± 0.0022
2	0.9324 ± 0.0070	0.7461 ± 0.0257	0.4751 ± 0.0318	0.9933 ± 0.0035

[a] Values represent mean ± SD (n = 3).

Table 3. Higuchi Release Rate Constant of Betanin from Chitosan Microparticle (n = 3)

Chitosan Concentration (%)	K[a] ($mg/min^{1/2}$)
0.5	6.37 ± 0.190
1	7.02 ± 0.293
2	3.62 ± 0.1904

[a] Calculated as betanin. Values represent mean ± SD (n = 3).

The t-test statistical analysis on the betanin release profile from the microparticles of beetroot extract revealed that increasing concentration of chitosan as an MP matrix from 0.5 to 1% *w/v* did not have a significant effect on the release rate of betanin from MP ($p > 0.05$), whereas increasing chitosan to 2% *w/v* had significantly reduced in betanin release rates from chitosan microparticle compared to chitosan 0.5 and 1% ($p < 0.05$).

4 Conclusion

The chitosan matrix used for beetroot MP preparation (0.5%, 1%, and 2% *w/v*) affected the encapsulation efficiency and the release of betanin from MP. The chitosan 0.5% and 1% showed the increasing value of encapsulation efficiency and release rate, although it was statistically not significant. Meanwhile, at the chitosan concentration of 2%, the release of betanin reduced. In conclusion, using a higher amount of chitosan could diminish the encapsulation efficiency and the rate of betanin release from chitosan microparticles as a high concentration of chitosan induced high viscosity during preparation.

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