Microencapsulation of Riboflavin (Vitamin B₂) using Alginate and Chitosan: Effect of Alginate and Chitosan Concentration upon Microcapsule Diameter

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Abstract. Riboflavin (Vitamin B₂) plays an important role in the development of human body tissue, the production of red blood cells and helps release energy derived from protein. Riboflavin cannot be produced and stored by the human body therefore must be supplied outside via food. Riboflavin is very sensitive and unstable to environmental influence such as light and reductor. One of technology for maintaining the stability of riboflavin is microencapsulation which is composed of polymer matrix containing riboflavin. Alginate was chosen as a polymer matrix because of riboflavin's absorption ability, biodegradable, biocompatible and non-toxic. The matrix polymer is strengthened by coating it with chitosan. The layer is then reinforced by crosslinking with glutaraldehyde. This research aims to study the microencapsulation process of riboflavin with alginate and chitosan. This research also studied the effect of alginate and chiton concentrations upon the diameter of microcapsules. The results showed that microencapsulation of riboflavin with alginate and chitosan can be done. The increase in chitosan concentration will reduce the size of microcapsules diameter. The concentration of alginate does not significantly affect the size of microcapsules diameter. Average diameter microcapsule range 380 – 610 µm, smaller than other research studies.

Keywords: riboflavin, microencapsulation, alginate, chitosan.

1. Introduction

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine) is a watersoluble vitamin present in a wide variety of foods. It can be crystallized as orange-yellow crystals [1]. Fig. 1 shows the chemical structure of riboflavin.

Figure 1. Chemical structure of riboflavin.

Riboflavin plays an important role in breaking down proteins, fats and carbohydrates. It play a significant role in maintaining energy supply. Riboflavin helps in production...
red blood cells and maintaining human tissues. The human body cannot provide riboflavin itself therefore it must be supplied from outside through dairy food.

One of the obstacles for supplying riboflavin to the human body is the highly sensitive and unstable nature of riboflavin to environmental influences such as light and reducing agents. One way to maintain the riboflavin stability is by encapsulation technology composed of a matrix of polymers containing riboflavin.

Suitable matrix polymers for riboflavin encapsulation must be biodegradable, biocompatible and non-toxic, one of the material that meets the requirements is alginate. Alginate is an anionic polysaccharide compound which is composed of two basic units (blocks), $\alpha$-L-guluronic acid (G) and $\beta$-D-mannuronic acid (M) which are linearly connected with connections 1-4. There are 3 forms of alginate arrangement, namely the arrangement of blocks G-G, M-M, and M-G as seen in Fig. 2.

![Figure 2. Blocks arrangement of alginate, G-G, M-M, and M-G.](image)

Na-alginate solution can turn into sol-gel form when cross-linked with divalent or polyvalent cations such as Ca$^{2+}$ and Zn$^{2+}$ [2] but Ca$^{2+}$ is more commonly used because it can bind to guluronic acid groups to form tissues with an egg-box structure [3]. These properties make sodium alginate has a great ability as a carrier of active compounds such as riboflavin [4]. Na-alginate matrix has a weakness in releasing the active compound easily so it is not suitable to be used as a control release of active compounds [5].

To overcome the limitations of Na-alginate matrix for maintaining the release of active compound, the alginate matrix is coated with chitosan. Chitosan is a linear (semi-crystalline) polysaccharide composed of units of N-acetyl D-glucosamine and D-glucosamine units. Chitosan structure can be seen in Fig. 3.

![Figure 3. Chemical structure of chitosan.](image)

Chitosan is biodegradable and biocompatible also, that’s why chitosan was selected for Na-alginate matrix coating. Strong electrostatic interactions between amino groups in chitosan and carboxylate groups in alginites will form a chitosan / alginate complex which is able to keep the release of an active compound. This chitosan / alginate complex is used to protect microcapsules in the encapsulation process and regulate the release of an active compound [6], [7]. Chitosan can be strengthen with glutaraldehyde by cross-linking process.

Microencapsulation is a technique of isolating active ingredients by forming micro-sized spherical material in which the active ingredient is protected by a layer. Some of the goals of
microencapsulation of vitamins according to Katouzan and Jafari [8] are protecting vitamins from external influences, regulating the release of vitamins, wrapping the unpleasant taste and odor of vitamins, and protecting the active life of vitamins.

The objective of this research was studying the microencapsulation riboflavin with Na-alginate and chitosan. This research also aims to study the effect of Na-alginate concentration dan chitosan concentration upon the microcapsule diameter.

2. Materials and Methods
Riboflavin was bought from Sigma-Aldrich. Chitosan and alginate were obtained from local store. Chitosan solution was obtained by dissolving it in acetic acid solution 1%. Na-alginate solution was made by dissolving it in aqueous solution. Calcium chloride and glutaraldehyde were used as crosslinking agent for Na-alginate and chitosan. Paraffin oil was used as emulsification medium and span 80 was used as surfactant agent.

Riboflavin (about 80 mg) was mixed in 30 mL Na-alginate solution. The mixed solution was dispersed in 70 mL paraffin oil which contained 0.3 mL span 80 using magnetic stirrer for 20 minutes. The dispersed solution then dripped slowly into 125 mL 1% CaCl₂ solution and stirred for 60 minutes. The microcapsules were filtered and washed. The microcapsules were then coated with chitosan solution for 15 minutes and filtered. The coated microcapsules were then cross-linked with 2% glutaraldehyde solution for 60 minutes. Then microcapsules were washed and dried.

The microcapsules diameter were determined manually using digital microscope. Microcapsule images were taken with a digital microscope then their size was determined using sizing software. The average diameter of the microcapsules is determined based on 120 microcapsule particles.

3. Results and Discussion
3.1. Microcapsules
The microcapsules form containing riboflavin can be seen in figure 4.

![Wet microcapsules](image1)
![dried microcapsules](image2)

Figure 4. The microcapsule form.

Fig. 4a showed the wet microcapsule before coated with chitosan and fig. 4b showed the microcapsule after coated with chitosan and dried. After coating with chitosan and dried, the microcapsule surface becomes wrinkled. This condition occurred due water reducing during the drying process. The yellow part in microcapsules indicate that riboflavin is encapsulated in Na-alginate matrix.

3.2. The effect of Na-alginate concentration upon microcapsules diameter
This research used Na-alginate solution in various concentration. The microcapsules diameter for various Na-alginate concentration can be seen in fig. 5. The figure showed that there are no significant effect of Na-alginate concentration upon microcapsule diameter.
3.3. The effect of chitosan concentration upon microcapsules diameter

This research used chitosan solution in various concentration. The microcapsules diameter for various chitosan concentration can be seen in figure 6.

Compared to Na-alginate solution, chitosan concentration has significant effect upon microcapsules diameter. The increase of concentration of chitosan used to coat the alginate microcapsules will reduce the size of the microcapsules formed. The large (more concentrated) chitosan concentration will cause the diffusion of water from alginate microcapsules into chitosan solution so that it will reduce the size of the microcapsules.

3.4. Comparison with other research studies

There have been several previous studies on the encapsulation of active material using alginate and chitosan. A comparison between these studies can be seen in Table 1.

Table 1. Comparison with other studies about encapsulation active material using alginate and chitosan

| Na-alginate concentration (%) | Average microcapsules diameter (µm) | Chitosan concentration (%) | Average microcapsules diameter (µm) |
|-------------------------------|-------------------------------------|-----------------------------|-------------------------------------|
| 1.5                           |                                     | 0                           |                                     |
| 2                             |                                     | 1                           |                                     |
| 2.5                           |                                     | 2                           |                                     |
| 3                             |                                     | 3                           |                                     |
| 3.5                           |                                     | 4                           |                                     |
| 4                             |                                     |                              |                                     |
### Active material | Methods | Size particle | Reference
--- | --- | --- | ---
riboflavin | dropwise alginate solution into the chitosan solution containing CaCl$_2$ using a hypodermic needle | About 1.4 mm | Bajpai and Tankhiwale [9]
drugs | dropwise alginate solution into the Chitosan solution containing CaCl$_2$ using a hypodermic needle | - | Li et.al. [10]
anti tubercular drug | dropwise alginate solution into the Chitosan solution containing CaCl$_2$ using a hypodermic needle | 670 – 830 µm | Sabitha et.al. [11]
insulin | dripping the alginate solution into the CaCl$_2$ solution using an extruder | 1 – 1.5 mm | Finotelli et.al. [6]
riboflavin | Dropwise alginate in oil phase emulsion into CaCl$_2$ solution | 380 – 610 µm | This research

4. Conclusions
The conclusions of the research showed that microencapsulation of riboflavin with alginate and chitosan can be done. The increase in chitosan concentration will reduce the size of microcapsules diameter and the concentration of Na-alginate does not significantly affect the size of microcapsules diameter. Average diameter microcapsule range 380 – 610 µm, smaller than other research studies

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