Microencapsulation of riboflavin (vitamin B₂) using alginate and chitosan: effect of alginate and chitosan concentration upon encapsulation efficiency

YC. Danarto¹,², Rochmadi¹, Budhijanto¹
¹Department of Chemical Engineering, Universitas Gadjah Mada, Indonesia
²Department of Chemical Engineering, Universitas Sebelas Maret, Indonesia

Corresponding author: yc.danarto@gmail.com

Abstract. Riboflavin (Vitamin B₂) plays an important role in the human tissue development, the production of red blood cells, and helps release energy by breaking down proteins, fats, and carbohydrates. Riboflavin cannot be produced in the human body and therefore must be supplied outside via dairy food. Riboflavin is very sensitive and unstable to environmental influences such as light and reduction agent. One of the technologies for maintaining the stability of riboflavin is microencapsulation which is composed of polymer matrix containing riboflavin. Na-alginate was chosen because it has properties such as biodegradable, biocompatibility, and non-toxic. Na-alginate also has a high loading capacity of riboflavin but Na-alginate is also easy to release riboflavin. Solving this issue, Na-alginate was coated with chitosan and the layer is then reinforced by crosslinking process with glutaraldehyde. The process of forming micro-sized particles was carried out by the emulsification ionic-gel method. This research aims to study the microencapsulation process of riboflavin with Na-alginate and chitosan. This research also studied the effect of Na-alginate and chitosan concentrations upon encapsulation efficiency. The research showed that the Na-alginate and chitosan concentrations had an effect on the encapsulation efficiency. The increase in the concentration of alginate and chitosan will make the encapsulation efficiency higher until it finally reaches the optimum point. Encapsulation efficiency will drop past that point. The optimum point for Na-alginate and chitosan concentrations are 3% and 2%, respectively.

1. Introduction
Riboflavin (Vitamin B₂) is a water-soluble vitamin that is needed for growth. It helps the body break down carbohydrates, proteins, and fats to produce energy, and it allows oxygen to be used by the body. Riboflavin is a 7,8-dimethyl-10-ribityl-isalloxazine molecule and orange-yellow crystals. The chemical structure of riboflavin can be seen in Figure 1. Riboflavin is flushed out daily and the human body cannot provide riboflavin itself; therefore, it must be supplied from outside through dairy food such as eggs, milk, nuts, soybeans, green leaf, etc.

The limitation for supplying riboflavin to the human body is due to the highly sensitive to environmental influences such as light and reducing agents; therefore, encapsulation of riboflavin by polymers is needed.
The polymers used for riboflavin encapsulation must be biodegradable, biocompatible and non-toxic. Alginate is the polymer that meet the criteria above. Sodium alginate is an anionic linear polysaccharide composed of alternating blocks of β-(1→4)-linked d-mannuronic acid (M) and α-(1→4)-linked l-guluronic (G) residues. The chemical structure of alginate can be seen in figure 2.

The negatively charged carboxyl groups in alginates cause the straight alginate chains to repel each other and this results in a stable aqueous solution. Alginate solutions can turn into sol-gel form when crosslinked with polyvalent ions such as Ca$^{2+}$ and Zn$^{2+}$ [1] but Ca$^{2+}$ is more commonly used because it can bind to guluronic acid groups to form tissues with an egg-box structure [1]. Rastogi et.al.[2] explain that Na-alginate has a great ability for loading active compounds such as riboflavin but according to Liu and Krishnan [3], Na-alginate has the disadvantage of easily releasing active compounds therefore is not suitable for control release active compounds.

Na-alginate needs to be maintained so it is not easy to release the active ingredient by coating it with a polymer such as chitosan. Chitosan is linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The chemical structure of chitosan can be seen in figure 3.

![Figure 1. Chemical structure of riboflavin.](image1)

![Figure 2. Chemical structure of Na-alginate with blocks arrangement M-G](image2)

![Figure 3. Chemical structure of chitosan](image3)
Similar to alginate, chitosan is biodegradable, biocompatible and anti-toxic. Amino groups in chitosan form strong electrostatic interaction with carboxylate groups in alginate generate chitosan/alginate complex which is able to withstand the release of active compounds. [4], [5]. Chitosan can be strengthened with glutaraldehyde by cross-linking process which is amino groups in chitosan react with aldehyde group in glutaraldehyde.

Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gasses in an inert shell, which in turn isolates and protects them from the external environment [6]. Some objectives of microencapsulation are protecting the sensitive substances from external environment, masking unpleasant odor or taste of substances, controlling the release of active substances, and safe handling the toxic material [7].

There are some research about microencapsulation of active substances with alginate and chitosan. These researches can be seen in table 1.

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

| Active substances | Methods | References |
|------------------|---------|------------|
| riboflavin       | dropwise alginate solution into the chitosan solution containing CaCl₂ using a hypodermic needle | Bajpai and Tankhiwale [8] |
| curcumine        | ionotropic pre-gelation followed by polication crosslinking | Das et.al. [9] |
| probiotic bact.  | dropwise the alginate solution into the CaCl₂ solution and then coated with chitosan solution | Trabelsi et.al. [10] |
| riboflavin       | Dropwise alginate in oil phase emulsion into CaCl₂ solution and then coated with chitosan solution | This research |

Encapsulation efficiency determines the number of active substances that can be contained in the microcapsules. The greater the encapsulation efficiency indicates that the microencapsulation process is also getting better because not much riboflavin is wasted during the process. Azevedo et.al. [11] studied the encapsulation efficiency of riboflavin in alginate-chitosan nanoparticles and it was about 55.9%. Jyothi et.al. [7] explained factors that influence the encapsulation efficiency such as:

- a. Solubility of polymer in organic solvent
- b. Solubility of organic solvent in water
- c. Concentration of the polymer
- d. Ratio of dispersed phase to continuous phase
- e. Rate of solvent removal
- f. Interaction between active substances and polymer
- g. Solubility of active substances in continuous phase
- h. Molecular weight of the polymer

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 encapsulation efficiency.

## 2. Experimental

### 2.1. Materials

Riboflavin was obtained from Sigma-Aldrich. Chitosan and alginate were obtained from local store. Chitosan solution was made 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.

### 2.2. Methods

Riboflavin (about 160 mg) was mixed in 60 mL Na-alginate solution. The mixed solution was dispersed in 140 mL paraffin oil which contained 0,6 mL span 80 using magnetic stirrer for 20 minutes. The dispersed solution then was 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.

2.3. **Encapsulation efficiency determination**

The encapsulation efficiency was determined using this equation

\[
EE\% = \frac{m_p}{m_i} \times 100\%
\]

which is

- \(EE\%\) = encapsulation efficiency
- \(m_p\) = mass of riboflavin in microcapsules
- \(m_i\) = initial mass of riboflavin

Initial mass of riboflavin \((m_i)\) is the amount of riboflavin used in the microencapsulation process. Mass of riboflavin in microcapsules \((m_p)\) is measured by extracting riboflavin in demineralized water and then determining its content in solution with a UV-Vis spectrometer.

3. **Results and Discussions**

3.1. **Microcapsules**

Microcapsules are formed by inserting riboflavin into Na-alginate solution and then microcapsules are formed by emulsion and crosslinking processes. These wet microcapsules can be seen in figure 4a. The microcapsules are then coated with chitosan, reinforced by crosslinking process using glutaraldehyde, and dried. This microcapsules can be seen in figure 4b. Compared to wet microcapsules, the dried microcapsules surface is more wrinkled. The average of microcapsules diameter range 380 – 610 µm.

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

**Figure 4.** The microcapsules form.

3.2. **The effect of Na-alginate concentration upon encapsulation efficiency**

This research used Na-alginate solution in various concentration (2%, 2.5%, 3% and 3.5%). Microcapsules do not formed at alginate concentrations below 2% and mixing riboflavin with alginate solutions is imperfect if alginate concentrations are above 3.5%. The encapsulation efficiency for various Na-alginate concentration can be seen in table 2. The greater in Na-alginate concentration will increase the encapsulation efficiency until it reaches a peak at a certain point and then decreases.

The ability to load and hold riboflavin in the alginate hydrogel is due to the formation of an "egg-box" structure during the crosslinking process with Ca\(^{2+}\) ions. The greater concentration of Na-alginate will cause more riboflavin that can be loaded and held in the Na-alginate hydrogel. This is related to the number of "egg-box" structures that are formed. But if the concentration of Na-alginate is too large it will cause the formation of "egg-box" structure will be inhibited because it is difficult to react Ca\(^{2+}\) ions with Na-alginate and riboflavin contained in Na-alginate will easily diffuse out. The optimum point is 3% Na-alginate concentration.
Table 2. Encapsulation efficiency for various Na-alginate concentration.

| Na-alginate concentration (%) | Encapsulation efficiency (%) |
|-------------------------------|-----------------------------|
| 2                             | 42.48                       |
| 2.5                           | 44.61                       |
| 3                             | 55.34                       |
| 3.5                           | 44.64                       |

Figure 5. Encapsulation efficiency for various Na-alginate concentration.

3.3. The effect of chitosan concentration upon encapsulation efficiency

This research used chitosan solution in various concentration (1%, 1.5%, 2%, 2.5%, and 3%). Chitosan solution is difficult to form at chitosan concentration above 3%. The encapsulation efficiency for various chitosan concentration can be seen in table 3.

Table 3. Encapsulation efficiency for various chitosan concentration.

| Chitosan concentration (%) | Encapsulation efficiency (%) |
|----------------------------|-----------------------------|
| 1.0                        | 44.61                       |
| 1.5                        | 45.29                       |
| 2.0                        | 55.70                       |
| 2.5                        | 28.06                       |
| 3.0                        | 27.60                       |
Figure 6. Encapsulation efficiency for various chitosan concentration.

The greater in chitosan concentration will increase the encapsulation efficiency until it reaches a peak at a certain point and then decreases. A large concentration of chitosan will make it easier to coat the alginate hydrogel and keep the riboflavin diffusion out. The concentration of chitosan that is too concentrated will have the opposite effect because it will push the diffusion of riboflavin out due to the large difference in the concentration of chitosan with alginate hydrogel. The optimum point is 2% chitosan concentration.

4. Conclusions
The research showed that microencapsulation of riboflavin with alginate and chitosan can be done. The results showed that the Na-alginate and chitosan concentrations had an effect on the encapsulation efficiency. The increase in the concentration of alginate and chitosan will make the encapsulation efficiency higher until it finally reaches the optimum point. Encapsulation efficiency will drop past that point. The optimum point for Na-alginate and chitosan concentration are 3% and 2% respectively.

Acknowledgement
This research was supported by Doctoral Disertation Grant by Kemenristek-Dikti Indonesia.

References
[1] Grant GT, Morris ER and Rees DA 1973 FEBS Letters 32(1) 195-8
[2] Rastogi R, Sultana Y, Aqil M, Ali A, Kumar S, Chuttani K and Mishra AK 2007 Int. J. Pharmaceutics 334 71-7
[3] Liu P and Krishnan TR 1999 J. Pharm. Pharmacol. 51 141-9
[4] Finotelli PV, Silva DD, Sola-Penna M, Rossi AM, Farina M, Andrade LR, Takeuchi AY and Rocha-Leao M H 2010 Colloids and Surfaces B : Biointerfaces 81 206-11.
[5] Polk A, Amsden B, De Yao K, Peng T and Goosen MFA 1994 Journal of Pharmaceutical Sciences 83(2) 178-85
[6] Ghosh SK 2006 Functional Coating (Willey Online Library) pp 1–28
[7] Jyothi NVN, Prasana, P M, Sakarkar SN, Prabha KS, Ramaiah PS and Srawan GY 2010 Journal of Microencapsulation 27(3) 187-97
[8] Bajpai SK and Tankhiwale R 2006 Reactive and Functional Polymers 65 1565-74
[9] Das RK, Kasoju N and Bora U 2010 Nanomedicine : Nanotechnology, Biology, and Medicine 6 153-60.
[10] Trabelsi I, Bejar W, Ayadi D and Salah RB 2013 Int. J. of Biological Macromolecules 61 36-42
[11] Azevedo MA, Bourbon AI, Vicente AA and Cerqueira MA 2014 Int. J. of Biological Macromolecules 71 141-6