



## Preparation of chitosan microspheres as carrier material to controlled release of urea fertilizer

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### ABSTRACT

This study aimed to investigate the effects of volume ratio in the continuous phase and the dispersed phase (CP/DP) and stirring speed on yield, water absorption, particle size, morphology surface, and cumulative release of nitrogen from the chitosan microsphere. The chitosan microsphere preparation method was emulsion cross-linking with glutaraldehyde saturated toluene (GST) as the cross-linking agent. Urea was mixed with chitosan solution and put into the oil. It was then stirred to form an emulsion. Glutaraldehyde saturated toluene (GST) was added slowly, followed by 2 ml glutaraldehyde, stirred continuously for 2 h. The formed chitosan microsphere was eventually washed and dried to be analyzed. The yield obtained was about 87.01%–96.71%, the water absorption was 79.77%–89.25%, and the diameter size of chitosan microspheres was from  $153.66 \pm 26.35 \mu\text{m}$  to  $179.39 \pm 34.95 \mu\text{m}$ . The chitosan microspheres morphology were good sphericity even though there were irregular size. Cumulative release analysis was obtained from 32.38%–37.69% by release mechanism such as anomalous behavior (non-Fickian kinetics)

### 1. Introduction

Indonesia is an agrarian country in which around 27.33% of its inhabitants work for agriculture (<https://www.antaraneews.com/berita/1223248/kebijakan-impor-beras-dan-ketahanan-pangan-indonesia>, 2020). The increase in population growth directly affects fertilizer needs to boost yield. Nitrogen is the primary nutrition needed for plant growth. Nitrogen is a composer of protein and chlorophyll in significant parts of the plant's body. Nitrogen in plants is also responsible for accelerating the growth, stimulating roots, increasing the quality of fruits, and controlling the whole growth (Hayatsu, 2014; Leghari et al., 2016).

Urea is a fertilizer that is mainly used because it contains high nitrogen (46%), and it is easy to handle. However, urea has some weaknesses. Firstly, the amount of nitrogen absorbed by the plant is quite low (around 30–35%). The rest of it disappears due to the leaching by rainfall and water flow. Urea hydrolysis changes quickly into

ammonium because of urease activity. Moreover, nitrogen can be quickly released into the air in the form of ammonia and disappears as nitrate, which turns to be an environmental and health issue (Guo et al., 2018; Maharani and Novan., 2017; Rekowski et al., 2020; Tong et al., 2018).

A number of innovations so far have focused on controlling the release of nutrients to increase the efficiency of nutrient absorption by plants. A controlled release of fertilizer can be achieved by wrapping fertilizer with polymer (biopolymer) or mixing organic or inorganic material as the composite. The controlled release of fertilizer is fertilizer layered with a polymer that is less sensitive to soil and climate, so the controlled release of fertilizer can predictably go on well (Jayanudin and Lestari, 2020; Trenkel, 2010). Moreover, the controlled release of fertilizer reduces soil and water toxicity, saves cost, power, time, and energy, and increases plant nutrient absorption.

The use of biopolymer as a urea fertilizer carrier has been observed in numerous studies. For example, Azeem et al. (2020) used tapioca starch

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modified with polyvinyl alcohol and citric acid as the wrapper using the rotary fluidized bed method to produce controlled-release coated urea granules. Before that, [Azeem et al. \(2016\)](#) formed urea fertilizer controlled release using a waterborne starch biopolymer modified with polyvinyl alcohol (PVA) in a fluidized bed. Another biopolymer used to wrap urea is PVA-glycerol-starch in the form of film ([Lum et al., 2013](#)). Moreover, [Phang et al. \(2020\)](#) did a study using alginate starch to control the release of urea fertilizer. The function of those materials is the same – wrapping urea fertilizer, so that the release can be controlled, and it can increase nutrient absorption by the plant. The present study focuses on using a chitosan as a urea fertilizer carrier to control the release.

Chitosan is a natural polymer not only used in agriculture, but also for medical, cosmetic, and biomedical purposes. In agriculture, it is used as a carrier for controlling the release of pesticide, herbicide, macro and micronutrient, and plant hormones. This is made possible not only due to its biocompatibility, biodegradability, and high permeability; it is also cost-effective, nontoxic, and able to form a film ([Cota-Arriola et al., 2013](#); [Kashyap et al., 2015](#); [Mujtaba et al., 2020](#)). A significant number of methods apply chitosan as a fertilizer carrier, and one of the methods used in this study was emulsion cross-linking, with glutaraldehyde saturated toluene (GST) as the cross-linking agent.

The previous emulsion cross-linking method that used chitosan cross-linked to GST was successfully conducted to wrap medicines ([Campos et al., 2013](#); [Jayanudin et al., 2019](#); [Ofokansi et al., 2013](#)). The emulsion cross-linking method includes cross-linking process between amine from chitosan with aldehyde from GST to form a chitosan molecule chain, which is directly converted into microsphere ([Dhakar et al., 2010](#); [Manjanna et al., 2010](#)). The advantage of this emulsion cross-linking method is that it can be used in the encapsulation process that dissolves each other, non-dissolve, for solid or liquid. It can also be used to produce the size of microparticle and nanoparticle ([Manjanna et al., 2010](#); [Mitra and Dey, 2011](#)).

The previous study by [Jayanudin and Lestari \(2020\)](#) used chitosan cross-linked with the glutaraldehyde solution as an NPK fertilizer carrier. Some weaknesses were identified, however: the formed microsphere stuck to each other, and its shape was irregular (chitosan microspheres did not show good spherical geometry). These weaknesses can be overcome by using the emulsion cross-linking method with GST as the cross-linking agent in this study. The initial stage of the emulsion cross-linking technique is the formation of droplets in the emulsion. In addition to the effect of the chitosan concentration, another essential factor is the volume ratio of the continuous phase to the dispersed phase (CP/DP). CP refers to vegetable oil, while DP is chitosan as urea coating.

The volume ratio of CP/DP affects the rate of compaction of microspheres to produce chitosan microspheres with a smooth surface. The CP/DP volume ratio also influences the porosity of the microspheres, which has an important effect on the release characteristics of the core material from the microspheres and is associated with the initial burst effect ([Jyothi et al., 2010](#); [Mao et al., 2008](#)). Therefore, the volume ratio of CP/DP was the focus of this study. Another parameter to consider was the stirring speed for the preparation process of urea-loaded chitosan microspheres. This stirring speed affects the diameter size of chitosan microspheres. This study aimed to find out ratio changes in the dispersed phase and the continuous phase and how the changes of stirring speed influenced yield, particle size, morphology surface, and cumulative release of nitrogen from the chitosan microsphere.

## 2. Materials and methods

### 2.1. Materials

This study used a urea fertilizer from PT. Pupuk Sriwidjaja, 25% (v/v) glutaraldehyde solution from Merck, 96% (v/v), technical toluene from CV. Tri Jaya Dinamika, chitosan (DD = 87.20% and viscosity 37.10 cps) from PT. Biotech Surindo, vegetable oil from PT. Sarwana

Nusantara, glacial acetate acid from Merck, and petroleum ether and technical grade of *n*-hexane from CV. Labora

### 2.2. Preparation of glutaraldehyde saturated toluene (GST)

Glutaraldehyde saturated toluene (GST) was prepared by mixing glutaraldehyde solution in concentration of 25% (v/v) with toluene. The volume ratio of glutaraldehyde and toluene was 1:1. The mixture was then stirred at a speed of 500 rpm for 3 h. The mixture was then set aside for 24 h before to take the highest layer as glutaraldehyde saturated toluene (GST) ([Jayanudin et al., 2019](#)).

### 2.3. Preparation of urea fertilizer-loaded chitosan microsphere

Chitosan microsphere as the urea fertilizer carrier was prepared by using the emulsion cross-linking method, referring to [Jayanudin et al. \(2019\)](#). Chitosan was first dissolved in glacial acetate acid 1% (v/v) to produce chitosan solution in a concentration of 4% (w/v). An amount of 5 g urea fertilizer was added to the 50 mL chitosan solution and stirred until dissolved. Meanwhile, vegetable oil was prepared with various volumes to get ratios 4:1, 6:1, and 8:1 between vegetable oil and chitosan solution. The mixture of urea and chitosan solution was added slowly into vegetable oil stirred at a speed of 10,000 rpm, 15,000 rpm, and 20,000 rpm for 1 h to form an emulsion. About 20 mL GST was then added into the water-in-oil emulsion (the emulsion of chitosan solution in oil). The addition of GST was done in 4 steps. In each of the steps, drops of GST was added gradually into the emulsion and stirred. Following GST, 2 ml glutaraldehyde solution in a concentration of 25% (v/v) was added. The mixture was then stirred for another 2 h. After the process was finished, the chitosan microsphere was dried in an oven with a temperature of 65 °C. The yield, water absorption, and the surface morphology of the chitosan microsphere were finally analyzed, and the cumulative release of nitrogen from the chitosan microsphere was determined.

### 2.4. Yield

The yield from the urea fertilizer microcapsule was determined by counting the weight ratio of the urea fertilizer microsphere with the weight of the chitosan and urea fertilizer mixture, as shown in [Eq. \(1\)](#).

$$\% \text{ Yield} = \frac{\text{weight of urea fertilizer microsphere}}{\text{total weight of chitosan and urea fertilizer mixture}} \times 100\% \quad (1)$$

### 2.5. Measuring water absorption

In analyzing the water absorption from urea fertilizer loaded chitosan microsphere, this study modified the method used in [Liang and Liu \(2006\)](#). 1 gr dried urea fertilizer microcapsule was soaked in 50 mL water and left for 1, 3, 7, 14, 21, and 30 days. After that, the chitosan microsphere was filtered and weighed. The water absorbed in the dried urea microcapsule was counted using [Eq. \(2\)](#).

$$DA = \frac{M}{M_0} - 1 \quad (2)$$

in which *M* refers to the weight of the urea microcapsule that absorbs water, while *M*<sub>0</sub> refers to the dried urea microcapsule.

### 2.6. Characterization of urea fertilizer-loaded chitosan microsphere using scanning electron microscopy (SEM)

The morphology of urea-loaded chitosan microsphere was analyzed using SEM from JEOL type JSM-6510LA. The chitosan microsphere was coated with platinum. The resolution of high vacuum (HV) was 3.00 nm (30 kV) with a low vacuum of 4.00 nm (30 kV), and the tension acceleration was between 0.50 kV and 30 kV.

**Table 1**

Experimental design and factor levels in actual values for the two constituents that affect the yield and diameter size of chitosan microspheres.

Factors	Name	Levels		
		-1	0	1
X <sub>1</sub>	Ratio of CP/DP	4: 1	6: 1	8: 1
X <sub>2</sub>	Stirring speed	10,000	15,000	20,000
Run	Factors	Yield (%)	Diameter size of chitosan microsphere (μm)	
	Volume ratio of CP/DP	Stirring speed (rpm)		
1	4: 1	20,000	87	163.08 ± 45.01
2	6: 1	20,000	91.33	159.32 ± 41.17
3	8: 1	20,000	96.71	153.66 ± 26.35
4	8: 1	10,000	95.86	179.39 ± 34.95
5	8: 1	15,000	94.83	169.01 ± 32.59
6	8: 1	20,000	96.71	153.66 ± 26.35
7	6: 1	20,000	91.33	159.32 ± 41.17
8	6: 1	20,000	91.33	159.32 ± 41.17
9	6: 1	20,000	91.33	159.32 ± 41.17

## 2.7. Release test of urea fertilizer in water medium

This test was used to determine the amount of urea fertilizer (nitrogen level) release from the chitosan microsphere. An amount of 0.20 g dried urea-loaded chitosan microsphere was soaked in 50 mL water for 1, 3, 7, 14, 21, 30 days at room temperature. The amount of urea released was determined by counting the nitrogen level using Nessler's reagent.

## 2.8. Release kinetics

The release kinetics of the nitrogen level from the chitosan microsphere was then calculated by using the following Eq. (3)–(4) based on the study conducted by Dash et al. (2010) and Dozie-Nwachukwu et al. (2017).

### 1 Higuchi model

$$\frac{M_t}{M_\infty} = k_H t^{1/2} \quad (3)$$

### 2 Korsmeyer-Peppas model

$$\frac{M_t}{M_\infty} = k_{K-P} t^n \quad (4)$$

in which  $\frac{M_t}{M_\infty}$  is the cumulative release of nitrogen;  $k_H$ ,  $k_{K-P}$  are the constants of Higuchi and Korsmeyer–Peppas model, and  $n$  indicates release mechanism.

## 2.9. Experimental design

Several factors, such as the volume ratio of the continuous phase and dispersed phase (CP/DP) ( $X_1$ ) and stirring speed ( $X_2$ ), affect the preparation process of chitosan microspheres filled with urea. The design to determine the yield and diameter size of chitosan microspheres is influenced by the volume ratio of CP/DP and stirring speed. Each independent variable was tested at three levels (-1, 0, 1), and nine experiments were carried out according to the statistical design. Experimental design using an actual, high, and low level of the factors was shown in Table 1.

Eq. (5) shows the second-order response function for the three quantitative factors.

**Table 2**

The yield of urea-loaded chitosan microsphere.

Ratio of the continuous phases: dispersed (volume ratio of chitosan solution and oil)	Stirring speed (rpm)	Yield (%)
4: 1	20,000	87.00
6: 1	20,000	91.33
8: 1	20,000	96.71
8: 1	15,000	94.83
8: 1	10,000	95.86

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \quad (5)$$

Where  $X_1$  and  $X_2$  are the levels of the independent variables, while for  $\beta_0$ ,  $\beta_1$ , and  $\beta_3$  are the specified regression coefficients.

## 3. Results and discussion

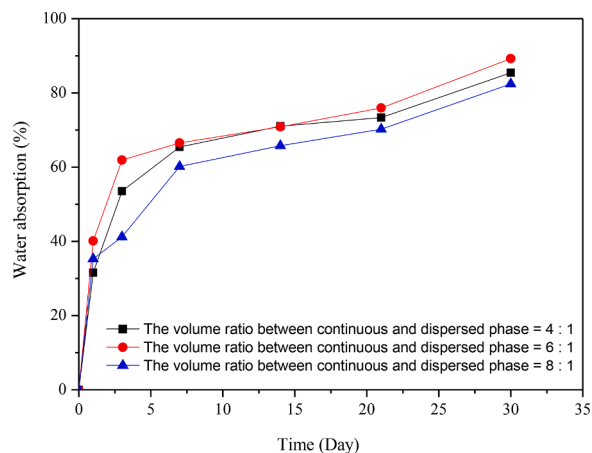
### 3.1. Determining yield of urea-loaded chitosan microsphere

The low efficiency of conventional urea fertilizer was caused by urea fertilizer that is quickly dissolved due to the water flow in the soil. For the efficiency of nutrient absorption by the plant to increase, the conventional urea fertilizer can be changed into urea fertilizer controlled release. Chitosan was used as a urea fertilizer carrier in the form of a microsphere.

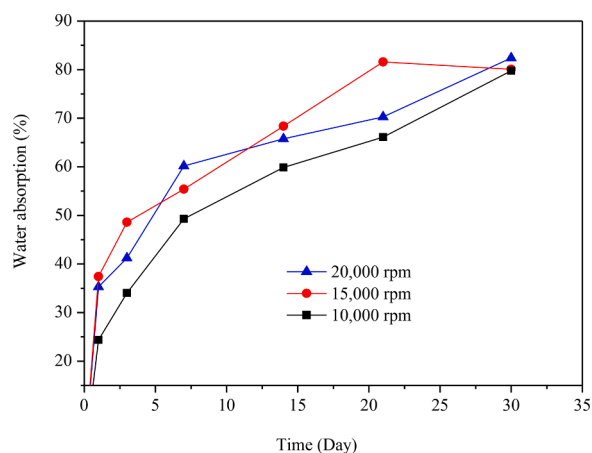
The method employed is emulsion cross-linking with glutaraldehyde saturated toluene (GST) as the cross-linking agent. The cross-linking reaction between chitosan and glutaraldehyde involves formation from the Schiff base. Schiff base bond was formed through a reaction between a functional group of aldehyde and  $-NH_2$  from chitosan (Zhao, 2012). The interaction between the amine functional group (chitosan) and aldehyde (GST) affects the yield value. Table 2 shows the yield of urea loaded-chitosan microsphere

Table-2 shows the influence of different ratios in the continuous phase and the dispersed phase (CP/DP) towards the formation process of emulsion and the influence of varying stirring speeds towards yield in the chitosan microsphere that contains urea. The increase of ratio in the continuous phase and the dispersed phase increased (CP/DP) the yield in the chitosan microsphere. The increase of continuous phase indicates the increase of volume in vegetable oil. The emulsion formed in this study was water in oil (W/O), in which the chitosan solution was coated by vegetable oil. The higher the volume in the continuous phase (vegetable oil), the more chitosan solution veiled by oil in the form of droplets. Afterward, the droplets were cross-linked with the GST to form a chitosan microsphere. The same result was also found in the study conducted by Devrim and Canefe, 2006, in which the decrease in the continuous phase caused the microsphere to merge, hence microsphere yield becoming lower. The changes in stirring speed produced no significant effect as the difference was only 1%. The variation of stirring speed, however, resulted in fluctuating yields in the chitosan microsphere

The influence of stirring speed towards yield in the chitosan microsphere is also noticeable in Table 2. The yield produced from the stirring speed of 15,000 rpm was lower than that from the 10,000 rpm, while the yield produced from a stirring speed of 20,000 was the highest. The finding suggests that the increase of stirring speed eased the droplet formation and increased emulsion stability made possible by decreased interface tension. The emulsion stability influenced the yield in the chitosan microsphere for an apparent reason. When the droplet was dripped, GST did not crack or combine with other droplets until the droplets were solidified to form a microsphere. This explains why the stirring speed of 20,000 rpm produced the highest yield compared to 10,000 rpm and 15,000 rpm.



**Fig. 1.** Analysis of the water absorption capacity of the urea-loaded chitosan microspheres. The examined chitosan microsphere is based on the change of volume ratio in the CP/DP over chitosan solution in a concentration of 4%, stirring speed of 20,000 rpm, and 5 g urea.



**Fig. 2.** The effect of stirring speed on the water absorption of the urea-loaded chitosan microsphere. The examined chitosan microsphere is based on the change of volume ratio in the continuous phase and the dispersed phase over chitosan solution in a concentration of 4%, stirring speed of 20,000 rpm, and 5 g urea.

### 3.2. Determining water absorption of urea-loaded chitosan microsphere

Water absorption is needed to determine the ability of the chitosan microsphere in collecting water, which then releases nitrogen from the microsphere. Fig. 1 shows water absorption from the chitosan microsphere influenced by varying volume ratios in the continuous phase and the dispersed phase (CP/DP).

Fig. 1 shows that the changes in volume ratio in the CP/DP affected the water absorption from the chitosan microsphere. No significant difference was identified between the figures of water absorption at ratio 4:1 and ratio 6:1, as the figures during the release time (1–30 days) were almost the same. The significant change happened at the volume ratio 8:1 in the CP/DP, where the value of water absorption was smaller than that at the ratio 4:1 and 6:1. The increase of oil volume accelerated the stiffening process of the microsphere (Jyothi et al., 2010; Mehta et al., 1996). As a result, the chitosan microsphere formed at the volume ratio 8:1 was stronger than that formed at the volume ratio 4:1 and 6:1 with the same stiffening time. The maximum water absorption from the chitosan microsphere with 30 min immersion time reached 89.24% at the volume ratio 6:1 in the CP/DP, while the lowest water absorption was identified at the volume ratio 8:1 (82.43%).

**Table-3**

The effect of the volume ratios between the continuous phase and the dispersed phase (CP/DP) and the stirring speed on the size of the chitosan microsphere.

Volume ratio of CP/DP	Size of chitosan microsphere ( $\mu\text{m}$ )	Stirring speed (rpm)	Size of chitosan microsphere ( $\mu\text{m}$ )
4: 1	163.08 $\pm$ 45.01	10,000	179.39 $\pm$ 34.95
6: 1	159.32 $\pm$ 41.17	15,000	169.01 $\pm$ 32.59
8: 1	153.66 $\pm$ 26.35	20,000	153.66 $\pm$ 26.35

Mean  $\pm$  SD,  $n = 100$ .

Fig. 2 shows that the process of changing the stirring speed did not have a significant effect on the water absorption of the chitosan microsphere. It is noticeable that the water absorption from the chitosan microsphere with the stirring speed of 20,000 and 15,000 rpm, when it increased, was quite unstable. With the stirring speed of 10,000 rpm, however, the water absorption tended to be more stable during 1–30 days of immersion. An insignificant difference in the figures of water absorption was identified on the 30th day when the figures were almost the same. The effect of stirring speed on the water absorption of microsphere chitosan is related to the absorption area - the higher the stirring speed, the smaller the size of the chitosan microsphere. Therefore, the area of water absorption is wider, and the capacity of water absorption is also higher. Thus, the area of water absorption with the stirring speed of 15,000 rpm and 20,000 rpm was smaller than that with the speed of 10,000 rpm. As a result, the water absorption of the chitosan microsphere prepared with the stirring speed of 20,000 rpm and 15,000 rpm is greater than that with the speed of 10,000 rpm. The lowest score of water absorption identified was 79.77% at 10,000 rpm, while the highest one was 82.43% at 20,000 rpm.

### 3.3. Diameter size of urea-loaded chitosan microsphere

Preparation of urea-loaded chitosan microsphere with the emulsion cross-linking method was successfully conducted, and the results produced various sizes of chitosan microsphere. The diameter of the chitosan microsphere was determined by using a digital microsphere observing 100 chitosan microspheres. The changes in the size of the chitosan microsphere affected by varying volume ratios between the continuous phase and the dispersed phase (CP/DP) are displayed in Table-3.

The average diameter change of the chitosan microsphere in Table 3 shows that the greater the volume in the continuous phase (oil), the smaller the diameter of the chitosan microsphere. Similarly, the increase of stirring speed resulted in the decline in the average diameter of the chitosan microsphere. The highest average diameter of the chitosan microsphere was obtained from the volume ratio 1:8 with the stirring speed of 10,000 rpm (179.393  $\pm$  34.95  $\mu\text{m}$ ). In contrast, the smallest average diameter was identified from the volume ratio 1:8 with the stirring speed of 20,000 rpm (153.658  $\pm$  26.35  $\mu\text{m}$ ).

It is still unclear how the volume ratio of CP/DP affects the size of the chitosan microsphere since similar studies in the past demonstrate different results. One study concludes that the increase in volume in the continuous phase leads to an increase in microsphere size. However, other studies reported that the volume ratio of CP/DP has no influence on the size of the microsphere (Heiskanen et al., 2012). In this study, the decrease in the size of the chitosan microsphere as a result of the increase in volume ratio in the continuous phase might increase the formation of emulsion droplets, and the high stirring speed level increased emulsion stability and decreased emulsion droplets. The results of this study were different from that of Jeffery et al. (1993) and Gabor et al. (1999) in that the increasing volume in the continuous phase decreased the size of the particle due to the increasing number of droplets collision, which then increased droplets coalescence and the average size of droplets (Heiskanen et al., 2012).

The size of the chitosan microsphere was also affected by stirring



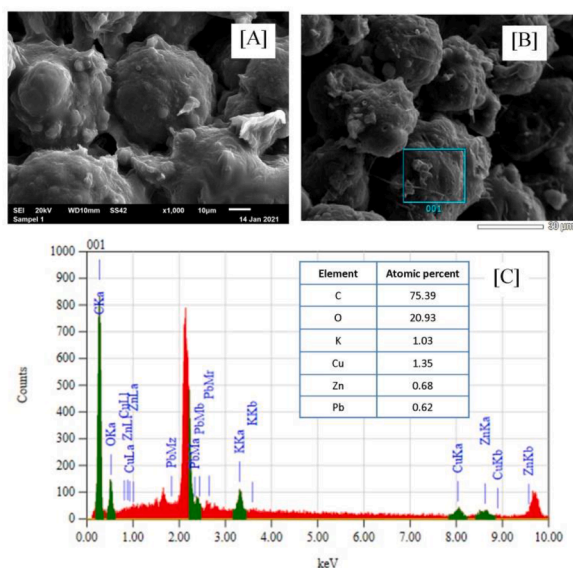


Fig. 3. SEM image of urea-loaded chitosan microspheres. [A] Urea loaded chitosan microsphere at a magnification of 1000x, [B] SEM-EDX image for chitosan microspheres, and [C] Quantitative analysis of urea loaded microsphere chitosan shown by atomic percent.

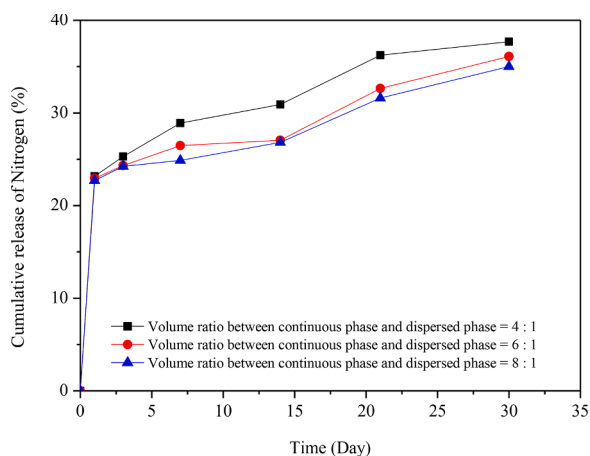


Fig. 4. The change of nitrogen released cumulative from the chitosan microsphere by the change of the volume ratio between the continuous phase and the dispersed phase (CP/DP).

speed. The size of the urea-loaded chitosan microsphere decreased due to an increase in stirring speed. This study assumes that the size of the chitosan microsphere is the same as that of emulsion droplets, so the stirring speed has a direct influence on the size of the chitosan microsphere. In other words, the stirring process will affect the value of interface tension – the faster the stirring speed, the smaller the interfacial tension. As a result, the size of emulsion droplets produced is smaller, which leads to the small size of the chitosan microsphere.

### 3.4. Morphological analysis of urea-loaded chitosan microspheres

Morphology analysis using a scanning electron microscope (SEM) of the urea-loaded chitosan microsphere is shown in Fig. 3.

Results of analysis using SEM showed that chitosan microspheres were good sphericity with smooth surfaces even though there were some irregular size diameters of microspheres. The perfectly spherical chitosan microspheres can be caused by the slow and uniform cross-linking of the droplets in the emulsion, especially on the surface (Thanoo et al.,

1992). These results were almost the same as those reported by Jayanudin et al. (2019) and Thanoo et al. (1992) in that SEM images showed that there were small lumps on the surface of the microspheres. This shape can be caused by the uneven cross-linking process between the chitosan layers in the emulsion droplet with GST. SEM-EDX analysis in Fig. 3C shows that the elements contained in chitosan microspheres filled with urea had the largest compositions, including oxygen (O) and carbon (C), which were 20.93% and 75.39% derived from chitosan and glutaraldehyde. Meanwhile, other compositions such as potassium (K), copper (Cu), zinc (Zn), lead (Pb) may be impurities from chitosan, glutaraldehyde, and materials for washing chitosan microspheres (hexane and petroleum ether).

### 3.5. Determining nitrogen release from chitosan microsphere

#### 3.5.1. The effect of volume ratio between the continuous phase and the dispersed phase (CP/DP)

The number of nitrogen released from the chitosan microsphere was determined by calculating the cumulative release. Fig. 4 shows the effect of the volume ratio of the CP/DP over the cumulative release of nitrogen from the chitosan microsphere.

Nitrogen released from the chitosan microsphere was affected by the volume ratio of the CP/DP. As a fertilizer, urea as a nitrogen supplier was absorbed in a cross-linked matrix between the amine group of chitosan and the aldehyde group of GST. The nitrogen release began with the chitosan microsphere absorbing water, followed by the controlled release of nitrogen. Fig. 4 shows that the highest grade of cumulative release was gained at the volume ratio 4:1 in the CP/DP (37.69%), while the lowest grade was at the volume ratio 8:1 (35.01%) after 30 days.

The increase of the volume ratio between the continuous phase and the dispersed phase (CP/DP) contributed to the decrease in the cumulative release of nitrogen and vice versa. The volume of the continuous phase affected the solidification process. The high volume accelerates the solidification process. Consequently, compared to that produced at the smaller volume, the microsphere produced at the higher volume in the continuous phase was harder. The low volume of continuous phase escalated the microsphere's porosity, which, in turn, widened the surface area (Jyothi et al., 2010; Mehta et al., 1996).

Porosity decreases with increasing volume ratio of CP/DP. Porosity has an important effect on drug release characteristics and was related to the initial burst effect (Mao et al., 2008). The volume ratio of CP/DP of 8:1 had a lower diffusion rate than the ratio of CP/DP of 4:1 and 6:1. The same phenomenon was reported by Mao et al. (2008), where an increase in the volume ratio of CP/DP could reduce the cumulative release. The reason is that the lower volume ratio of CP/DP reduces the diffusion distance compared to microspheres prepared at a higher volume ratio of CP/DP, where the core material was distributed more homogeneously in the polymer matrix, leading to a faster release rate. The volume ratio of CP/DP ever used ranges from 20:1 to 300:1 (Jyothi et al., 2010; Mao et al., 2008). In this study, the volume ratio of CP/DP used is lower because it considers operational costs for purchasing oil as a continuous phase. Continuous phase volume reduction (vegetable oil) may reduce operating costs. Although the continuous phase volume of this study was reduced up to 5 times, it did not affect the preparation process of chitosan microspheres filled with chitosan.

The preparation process is complex and requires additional material costs as a urea matrix or coating. However, controlled-release urea fertilizer has many advantages such as increasing Nitrogen use efficiency (NUE), minimizing environmental pollution, especially nitrate leaching, evaporation of ammonia and nitrous oxides. Consequently, it reduces fertilizer application by about 20–30% for the same product, and it is more economical in terms of saving energy, time, and effort (Lawrencina et al., 2021; Xu et al., 2021). The increasing popularity and demand for slow/controlled-release fertilizers and the increasing variety of methods that can be used will reduce the selling price of

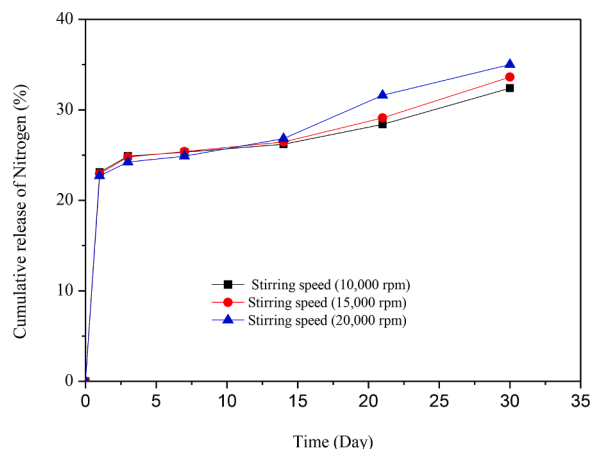


Fig. 5. The change of nitrogen released from the chitosan microsphere affected by stirring speed in the microsphere formation process.

slow-release/controlled release fertilizers.

### 3.5.2. Stirring speed effect

The changes in stirring speed for the preparation of the urea-loaded chitosan microsphere is related to the change in particle size. The effect of the changing diameter of the chitosan microsphere was then analyzed against the nitrogen released cumulative from the chitosan microsphere. The results are shown in Fig. 5.

The percentage of nitrogen released from the chitosan microsphere on the basis of the stirring speed is shown in Fig. 5. It is apparent that the change of cumulative release of nitrogen was insignificant in the first 14 days as the amount of nitrogen released was almost the same despite different stirring speeds. Nonetheless, after the first two weeks, the differences in nitrogen released were quite significant. At the speed of 20,000 rpm, the percentage of nitrogen released from the chitosan microsphere was higher than that at 15,000 rpm and 10,000 rpm. Moreover, the stirring speed in the preparation process of the chitosan microsphere affected the size of microsphere as is shown in Table 1. Smaller particle size was found to increase the nitrogen released since the surface area became wider (Trenkel, 2010). The same result was also reported by Dinarvand et al. (2003) and Capan et al. (1999) who stated that the smaller the particle size, the faster the release process.

Overall, from all parameters observed, the changes of volume ratio between the continuous phase and the dispersed phase up to 30 days the amount of nitrogen released was still relatively low. The highest cumulative release was gained from the volume ratio 4:1 between the continuous phase and the dispersed phase at a stirring speed of 20,000 rpm (3.69%). This figure is still relatively low given the notable capacity of the chitosan microsphere in absorbing water (79.77%–89.25%). This finding suggests that an enormous amount of nitrogen was still absorbed in the matrix of the chitosan cross-linked with glutaraldehyde.

The release rate of urea was influenced by the particle diameter's size of the chitosan microspheres. This is attributable to the change in the overall mass of the urea particles. In addition, the spread of urea through the coating layer was affected by the total amount of urea in the core. The larger the particle size, the more release time was obtained (Trinh et al., 2014). In order to increase the release time of urea, the size of the chitosan microspheres should be increased. Shaviv et al. (2003), however, stated that, technically, increasing the radius was more economical and feasible.

### 3.5.3. Release kinetics

Release kinetics is used to predict nitrogen released from the chitosan microsphere before being applied (Dash et al., 2010). The release kinetics equation used in this study was the Higuchi and Korsmeyer–Peppas model, as shown in the Eq. (3) and 4. The constants of

Table 4

Nitrogen released kinetics constants from urea-loaded chitosan microsphere.

The volume ratio between the continuous and the dispersed phase	Higuchi		Korsmeyer–Peppas		
	$K_H$	$R^2$	$K_{K-P}$	$n$	$R^2$
4: 1	1.18	0.85	1.738	0.52	0.87
6: 1	1.04	0.81	1.698	0.49	0.85
8: 1	0.99	0.81	1.738	0.49	0.85
Stirring speed (rpm)					
20,000	0.99	0.81	1.738	0.49	0.85
15,000	0.89	0.74	1.778	0.48	0.83
10,000	0.84	0.70	1.820	0.47	0.82

Table 5

Diffusional exponential ( $n$ ) and release mechanisms.

Diffusional exponent, $n$	Release mechanism
Spherical	
0.43	Fickian diffusion
$0.43 < n < 0.85$	Non-Fickian
0.85	Case II transport

release kinetics were determined by using the linear regression method for  $R^2$ . The Higuchi model describes the release in diffusion, where microspheres are not affected by the swelling process (Thanoo et al., 1992). Meanwhile, the Korsmeyer–Peppas model was used to determine the release mechanism of nitrogen by determining variable  $n$  and comparing it to the standard developed by Ritger and Peppas (1987). Constants and  $R^2$  values from the release kinetics are shown in Table 4.

Table 4 shows that, for all parameters of the study, the highest value of  $R^2$  was produced by the Korsmeyer–Peppas model, which means that it was the appropriate nitrogen released kinetics. The score of  $n$  from the Korsmeyer–Peppas model, as shown in Table 3, was 0.47–0.52. This score matched with Table 5 developed by Ritger and Peppas (1987) to determine the release mechanism.

Referring to Table-5, the nitrogen release mechanism from the chitosan mechanism is NonFickian, which indicates that the release mechanism (non-Fickian kinetics) related to the release of nitrogen was controlled by the process of diffusion and swelling (anomalous transport).

### 3.5.4. Development of a second-order polynomial regression model

The larger the particle size, the more release time was obtained (Trinh et al., 2014). In order to increase the release time of urea, the size of the chitosan microspheres should be increased. Shaviv et al. (2003) stated that, technically, increasing the radius was more economical and feasible. Design of Experiment (DOE) is used to obtain an empirical relationship between experimental results and predictions. The second-order polynomial equation (Eq. (5)) would help predict the interaction effect of the independent and dependent parameters. The resulting polynomial equation based on calculations using Table 1 data shown in Eq. (6) was affected by changes in the volume ratio of CP/DP and Eq. (7) for changes in stirring speed.

$$Y_1 = 89.875 + 4.43X_1 + 0.425X_1X_2 + 0.525X_1^2 + 1.455X_2^2 \quad (6)$$

$$Y_2 = 161.805 + 8.155X_1 - 12.865X_1X_2 - 0.95X_1^2 - 2.485X_2^2 \quad (7)$$

Where  $X_1$  is volume ratio of CP/DP,  $X_2$  is stirring speed.

Fig. 6 shows the values determined using Eqs. (6) and 7 and then plotted using actual versus predicted values for yield and diameter size of chitosan microspheres. The significance of the coefficients of the quadratic polynomial model (the second order) was conducted using ANOVA, and the results were shown in Table 6. The results of the analysis for the regression coefficient and p-value showed that linear, quadratic, and the interaction of the two CP/DP ratios and stirring speed had a significant effect on % yield and diameter of chitosan microspheres

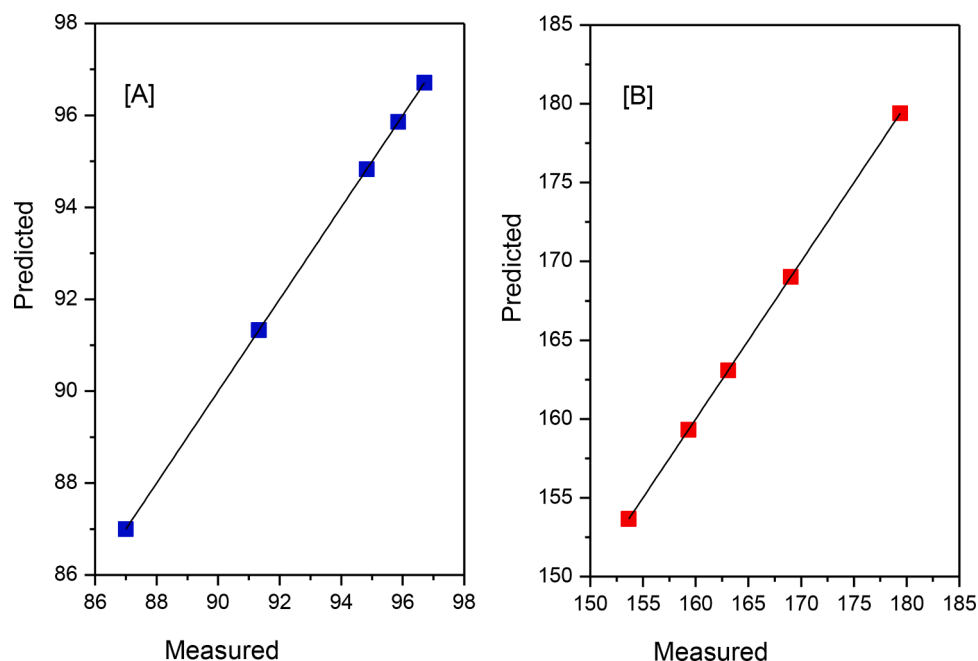


Fig. 6. Comparison between predicted and experimental results for volume ratio of CP/DP [A] and stirring speed [B].

Table 6

Determination of the regression coefficient for chitosan microspheres filled with urea fertilizer using analysis of variance.

Independent variable	Volume ratio of CP/DP		Stirring speed	
	Coefficient of regression	<i>P</i> -value	Coefficient of regression	<i>P</i> -value
Constant	89.875	$7.907 \times 10^{-69}$	161.805	$2.986 \times 10^{-66}$
Linear				
$X_1$	4.43	$1.268 \times 10^{-64}$	8.155	$4.381 \times 10^{-62}$
$X_2$	0	–	0	–
Quadratic				
$X_1^2$	0.525	–	-0.95	–
$X_2^2$	1.455	$8.243 \times 10^{-62}$	-2.485	$3.843 \times 10^{-59}$
Interaction				
$X_1X_2$	0.425	$8.422 \times 10^{-61}$	-12.865	$3.979 \times 10^{-63}$

( $P < 0.05$ ).

#### 4. Conclusion

In summary, the urea-loaded chitosan microsphere has been successfully prepared by the emulsion cross-linking method using glutaraldehyde saturated toluene (GST) as the cross-linking agent. The results of this study show that the change of the volume ratio between the continuous phase and the dispersed phase (CP/DP) affected yield, water absorption, and cumulative release of nitrogen. A higher volume ratio of (CP/DP) the yield gained from the chitosan microsphere and decreased the water absorption, the diameter size of the chitosan microsphere, as well as the cumulative release of nitrogen. On the other hand, the stirring speed made insignificant differences in the yield, the chitosan microsphere size, the water absorption, and the cumulative release of nitrogen. By determining the *n* score from the Korsmeyer–Peppas model, the discovered release kinetics was anomalous behavior (non-Fickian kinetics), which means that the mechanism of nitrogen release from the chitosan microsphere was controlled by diffusion and swelling.

#### CRediT authorship contribution statement

: Conceptualization, Formal analysis, Writing – review & editing. **Retno S.D. Lestari**: Conceptualization, Writing – original draft, Writing – review & editing. **Indar Kustiningsih**: Conceptualization, Writing – original draft, Writing – review & editing. **Dandi Irawanto**: Funding acquisition, Formal analysis, Writing – original draft. **Rozak Bahaudin**: Funding acquisition, Formal analysis, Writing – original draft. **Reynaldo L.A. Wardana**: Funding acquisition, Writing – original draft. **Fakhri Muhammad**: Funding acquisition, Writing – original draft. **Maulana Suyuti**: Funding acquisition, Writing – original draft. **Muhammad Luthfi**: Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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