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Research Article

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The use of biopolymer of poly(3-hydroxybutyrate) as matrix of urea slow release fertilizer

Akmal D.*, Monica R., Asiska P. D., Muslim S., Ben E. S. and Erizal Z.

Faculty of Pharmacy, University of Andalas, Padang, West Sumatra, Indonesia

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ABSTRACT

The use of biopolymer of poly (3-hydroxybutyrate) [(P(3HB)] as a matrix of urea slow release fertilizer has been carried out. The microencapsulation of urea was conducted using the solvent evaporation method. The ratio of urea-P(3HB) for formula 1, 2, and 3 were of 1-1, 1-2, and 1-3 respectively. Microcapsules were evaluated by Scanning Electron Microscope (SEM), Fourier Transform Infra Red (FTIR) spectroscopy, amount of urea in microcapsule, particle size distribution, release test, and kinetic release of active substance. Results showed that the biopolymer P(3HB) could be used as a matrix of urea slow release fertilizer. There was no chemical interaction between urea and P(3HB) during the process of microcapsules formation. The amount of urea in microcapsules in Formula 1, 2, and 3 were detected of 41.39%, 28.89%, and 24.47% respectively. The microcapsules formed the spherical shape with particle size distribution of urea in microcapsules was ranged from 11.4 to 607 µm. it is also observed that the release kinetics model of the active substance (urea) followed the Korsemeyer-Peppas equation. Statistical assay of One Way ANOVA showed that the use of P(3HB) affect the efficiency of release test significantly ($p<0.05$) which means that an increase in the concentration of P(3HB) as matrix could reduce the release of urea from the microcapsules.

Keywords: biopolymer, poly (3-hydroxybutyrate), matrix, urea, slow, release

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INTRODUCTION

Fertilizer plays an important role in enhancing the production and productivity of the agriculture. In the general, the Indonesian farmers use urea based-fertilizer [$CO(NH_2)_2$] (45–46%) as the primary nitrogen sources for supporting their food plant productions. Without urea based-fertilizer, the productivity of the plants could be declined [1. 2].

In facts, there was any obstacle found in plant cultivation. The fertilizer was not absorbed sufficiently by the plants. About 20-70% of the fertilizer either would be degraded or washed away in the soil water flow. This resulted in the inaccuracy of the fertilizers and the pollution due to the environment containing nitrogen [3]. So that, it's necessary to investigate another method to ensure the chemical elements in the fertilizer are released slowly and continuously in certain period of time to minimize the accident of water dragging.

An effort to enhance the effectiveness and efficiency of urea based-fertilizer could be performed by modifying the fertilizer into the matrix form which is able to release gradually in the soil [4]. This method of application is called as *Slow Release Fertilizer* (SRF). The advantage of such fertilizer is that the fertilizer will be available in the soil in the longer period of time compared to that by the conventional fertilizer (*fast release fertilizer*). This method also reduces the cases of evaporation, and water dragging [5].

The exertion to retard the nitrogen release from the fertilizer could reduce the case of environment pollution since the nitrogen in the form of nitrate that enter the waters is a source of water pollution. Nitrogen in the inorganic form (nitrate, nitric, and ammonia) is the indicator of the water pollution. Nitrification impacts the quality of environment

due to the oxidation of NH_4^+into NO_3^-which is easily soluble in water that cause the pollution in its nature. The high concentration of nitrate in water stimulates the grow of microbes, algae, plankton, water hyacinth, and another water plant due to the enrichment of water containing nitrate.

Microencapsulation is a method of thin layer used as the matrix either on the solid small particles or the drops. Microencapsulation is able to convert the liquid form into the solid one, protect the particles from the environment and control the characteristics release of the materials. One of method used to form microcapsules is emulsification of solvent evaporation. By this method, the process of microcapsule formation is initiated by separation of emulsion drops of dispersed phase into the mobile phase and form small drops. If the stirring procedure stopped, the forming microcapsules will be fall down to the base of the container. The technic of solvent evaporation is able to use in a wide variety of various core materials of liquid or solid forms. Either the soluble or the insoluble materials could be used as the core materials [6].

P(3HB) is a biopolymer that produced by bacteria such as Ralstonia euthopha and *Erwina sp* USMI-20 [7,8]. The biopolymer is known to have the biodegradable property and less toxic to the cells [9].

The American Society for Testing of Materials (ASTM) and *the International Standards Organization* (ISO) defined that the degradable polymer is the materials that alter its chemical structure in the certain condition significantly. The alteration caused any changes of its physical and mechanical characteristics. Biodegradable polymer is usually occured due to the action of microbes such as bacteria, fungi and algae [10].

In the present study, P(3HB) was used as the matrix of the microcapsules using the emulsification of solvent evaporation method and the observation of the kinetic release was performed later.

EXPERIMENTAL SECTION

Equipment and Materials

Tools used in the study were homogenizer *(IKA® RW Digital),* Fourier Transform Infrared (*Jasco*), UV-Vis spectrophotometer (UV-*1700 PharmaSpec,* Japan), analytical balance (*Shimadzu AUX 220,* Japan), Ocular microscope, mortar and stampher, sieve papers, drying cupboard.

The materials used were urea (Merck, Germany), biopolymer P(3HB) (Aldrick Chemical), dimethyl amino benzaldehyde (Merck, Germany), Polysorbate80 (PT. Brataco, Indonesia), Liquid paraffin (PT. Brataco, Indonesia), dichloromethane, n-hexane, distilled water, and soil containing water.

Raw Material Examination

The examination of raw material was performed based on the requirements stated in Indonesian Pharmacopeia 3rd.edition and United States Pharmacopeia XXXI such as apprearances and solubility . Then, the examination performed to P(3HB) were appearances of solubility, and Infra Red spectra identification [11].

Production Microcapsules Urea

The formula of slow release fertilizer of urea and P(3HB) as a matrix is shown in Table 1. P(3HB) was dissolved in a cup containing chloromethane and added the amount of urea (M_1). In another cup, the liquid paraffin in combination with span 80 was prepared (M_2). Then, M_1 was added into M_2 gradually and stirred under the velocity of 700 rpm until whole of dichloromethane evaporated. The formed microcapsules were collected by pouring out the excess liquid until the solid has settled at the bottom of container and washed with n-hexane four times. Later, the mass was filtered and dried in the drying cupboard.

Table 1. The formula of urea slow release fertilizer produced

Materials	Formula			
	F0	**Fl**	**F2**	**F3**
Urea (mg)	-	500	500	500
P(3HB) (mg)	500	500	1000	1500
Dichloromethane (mL)	20	20	20	20
Span 80 (mL)	1	1	1	1
Liquid parafin (mL)	100	100	100	100

Microcapsule evaluation

a. IR spectroscopy analysis

The absorbance of the microcapsules in the form of powder was measured using *Fourier Transform Infrared* (FT-IR) spectroscopy.

b. Particle size distribution

The particle size distribution of microcapsules yielded in the study was measured using optilab equipment. After being calibrated, the Optilab was connected to the ocular microscope and plugged into the computer. The samples were put on the object glass and then attached on the desk smear. The particles were observed and displayed on the computer screen and counted as 300 particles.

c. Determination of amount of urea in the microcapsule

The amount of 50 mg of microcapsules was quantitatively counted and grinded. Then, the mass was transfered into a 25 mL volumetric tube and dissolved with the distilled water, shacked and added 1 mL of Erlich reagent. The maximum wavelength of urea was measured using the UV-Vis spectrophotometer. Sampel for each formula was measured for three times.

d. *Scanning Electron Microscopy analysis*

The sample was attached to the sample holder and observed in the various magnification of SEM (Phenomm pro-X, Netherlands) under the condition of 5 kV and 12 mA.

e. *Release test*

Container of the testing medium was filled with 50 mL of soil containing water. An amount of urea microcapsules produced which was equal to 150 mg was added into the container. Then, 5 mL of the solution was taken at 10, 20, 30, 45, 60, 120, 240 and 360 minutes. The missing solutions were replaced with the test medium. The absorbance was measured using the UV-VIS spectrophotometer at the maximum wavelength. The measurement was conducted three times for each formula [12].

RESULTS AND DISCUSSION

The microcapsule formation was initiated by diluting the P(3HB) in the dichloromethane followed by adding the urea. In another container Span 80 was combined with liquid parafin and stirred using the homogenizer under 700 rpm. In the stirring process, urea and dichloromethane were dropped in the mobile phase carefully. The stirring process would yielded the emulsion consisted of urea and P(3HB). During the stirring process, the solvent would be evaporated which cause the breaking of the emulsion. This would produce the microcapsule particles in the mobile phase [6]. The stirring process was conducted for 5 hours continuously. The formed microcapsules were collected by pouring out the excess liquid until the solid has settled at the bottom of container and washed with n-hexane four times. The washing process aimed to ensure that the microcapsules were free of mobile phase. Later, the mass was dried using the vacuum oven under 70° C until whole mobile phase was evaporated.

FTIR spectroscopy of urea microcapsules showed that formed several spectrum referred to the functional group contained in the structure of urea as the active compound and P(3HB) as the matrix. There was no date functional group which implicated that there was no interaction between both molecules.

SEM analysis data (showed in Fig-1), indicated that urea microcapsule was formed in sphericalal shaped and there was the agregation which was predicted as the result of coagulation the small particle microcapsules. There were also less-spherical shaped microcapsules which produced by empty microcapsules. The particle sizes depend upon the velocity of stirring process. In the present study the velocity used was 700 rpm referred to the previous value in the similar study. This condition yielded the particle sizes ranged from 0 to 1.000 µm. The slower of stirring velocity would produce the bigger size and less-spherical shaped microcapsules. Inversely, the increasing of velocity would produce the smaller and less-spherical shaped particle [6, 13].

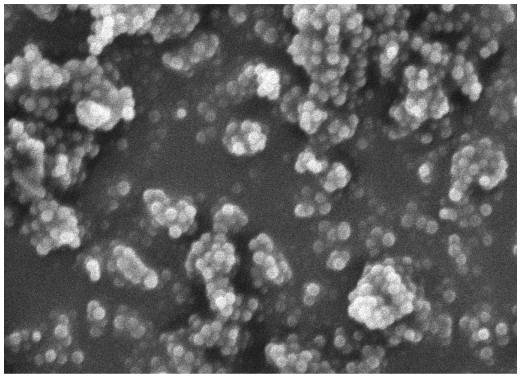

Fig 1. Scanning Electron Microscope (SEM) analysis of microcapsules containing urea of Formula 3

Particle size distribution of the microcapsules showed in Figure 2. Generally, the particle size ranged from 11.43 µm to 607 µm. The highest proportions of particle size of formula 1, 2, and 3 were ranged in similar value of 11,43 µm – 54 µm by the frequency of Formula 1, 2, and 3 were 58,33%, 66,34%, dan63,34% respectively. The previous study reported that the particle sizes distribution using the present method produced the particles in the range of 134.33 - 266 µm from Formula 1 and 3, weather Formula 2 yielded the particles ranged from 0-133 µm. The particle sizes yielded in the study fulfilled the requirements of microcapsule formation using the method of emulsification of solvent evaporation which had to be ranged in 5-5000 µm [6].

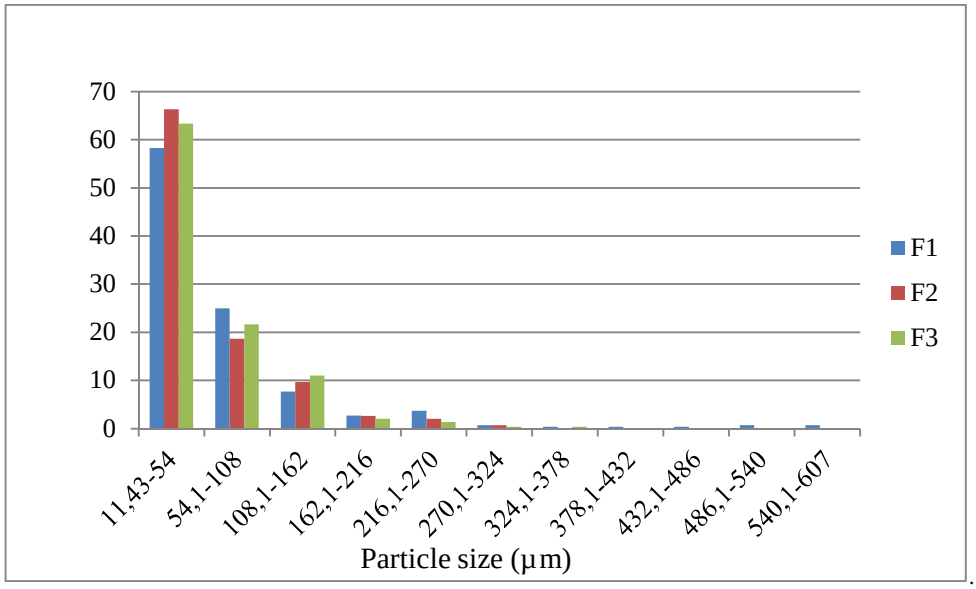

Figure 2. Graph of particle size distribution of urea microcapsules. F1 (Formula 1), F2 (Formula 1), F2 (Formula 1)

As general, the recovery value of active compound showed that the urea contained in the microcapsules was increase by increasing amount of P(3HB). It because ability of matrix to coat the core material would be increased by its increasing amount [13]. The recovery value of urea of Formula 1, 2 and 3 were 82.78 ± 0,68%, 86,65 ± 0,87%, and97,87 ± 0,45% respectively.

Figure 3 showed that the curve of release test of urea microcapsules in medium of soil containing water. The release test of urea microcapsules using P(3HB) as the matrix implicated that the urea releasing rate from the microcapsules was reduced. In this case, compared to Formula 1 and formula 2, Formula 3 showed the slower releasing rate. After 6 hours of application, the amount of active compound released from formula 1, 2 and 3 were 42.12 ± 0,60, 40,27 ± 0,48, 37,60 ± 0,32% respectively. It was indicated that the thicker layer on the microcapsule surface formed by P(3HB) will reduce the releasing rate of active compound. The reducing of urea releasing rate was caused by the hydrophobicity and insoluble property of P(3HB) which retarded the diffusion rate of water penetration. Finally, the time needed to release a certain amount of active compound was longer compared to the conventional fertilizer.

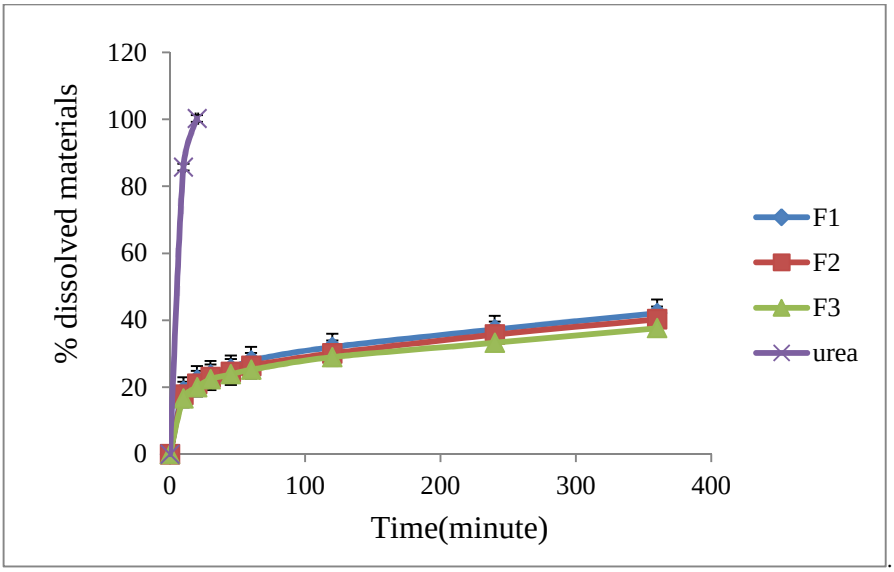

Fig 3. Curve of release test of urea microcapsules in medium of soil containing water. F1 (Formula 1), F2 (Formula 1), F2 (Formula 1)

Based on the average of release test efficiency obtained the efficiency value of formula 1, 2 and 3 were 33.38 ± 0,26%, 31,79 ± 0,11,30,01 ± 0,33% respectively. The result indicated that the increasing amount of P(3HB) as the coating agent decrease the release efficiency value. This also meant that there was reducing of microcapsule releasing rate. By using Statistic Assay, One way ANOVA, showed that the counted F was 134,351 with significant as 0.0001($p< 0.05$). This indicated that the P(3HB) use as the matrix affect the releasing rate significantly. Post Hoc Test also shown the significant value produced by each formula. This indicated that there was significant influence produced by the variation of matrix used in each formula.

Kinetic model determination of urea releasing rate of microcapsules was performed based on the zero order, one order, Higuchi, Langenbucher, and Korsemeyer-Peppas equation. It is also observed that the kinetic model of urea releasing rate of microcaspsules followed the Kosemeyer-Peppas equation which showed the highest linearity. It indicated that the kinetic of active compound released from microcapsules followed the diffusion law.

CONCLUSION

Our studies showed that the biopolymer P(3HB) could be used as a matrix of urea slow release fertilizer. There was no chemical interaction between urea and P(3HB) during the process of microcapsules formation. The amount of urea in microcapsules in Formula 1, 2, and 3 were detected of 41.39%, 28.89%, and 24.47% respectively. The microcapsules formed the spherical shape with particle size distribution of urea in microcapsules was ranged from 11.4 to 607 µm. it is also observed that the release kinetics model of the active substance (urea) followed the Korsemeyer-Peppas equation. Statistical assay of One Way ANOVA showed that the use of P(3HB) affect the efficiency of release test significantly ($p<0.05$) which means that an increase in the concentration of P(3HB) as matrix could reduce the release of urea from the microcapsules.

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