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Manufacture of a Slow Release Herbicide of Methyl-Metsulfuron using biopolymer of Poly (3-hydroxybutyrate) as Matrix

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ABSTRACT

The formulation of microcapsule of methyl-metsulfuron using biopolymer of poly(3-hydroxy-butyrate) as matrix of herbicide by solvent evaporation technique has been carried out. In the study, three types of the microcapsule formulation was used with the ratio between methyl-metsulfuron, and poly(3-hydroxybutyrate) of 2:1, 1:1, and 1:2 respectively. The particle distribution was measured using a calibrated microscope. Active substance release was determined by UV-Vis spectrophotometer. Results indicated that biopolymer of poly(3-hydroxy-butyrate) can be used as the matrix polymer for slow release preparations and influence the release of active substance of metsulfuron-methyl in the objects. It is also observed that the highest value of recovery test of active compound was formula 1 : 83.56%, followed by formula 2 : 71.22% and the last was formula 3 : 67.79% The kinetic model of active compound released from microcapsules with correlation coefficient of near by 1 and followed the zero order kinetic obtained from formula 1.

Keywords: Slow release, microcapsule**,** biopolymer, methyl-metsulfuron, poly(3-hydroxy-butyrate)

INTRODUCTION

The application of herbicide was very helpful in terms of increasing the agriculture productivities nowadays. Herbicide globally used as spraying liquid. The spraying application of herbicide could possibly cause many disadvantages such as negative impacts toward the environment when it is used during the winter since such herbicide is easily dissolved into water. Then, there comes the disease caused by herbicide poisoned water which human use.

Furthermore, the development of production method of herbicide is introduced in this study by controlling the release of its active compound in the form of micro-encapsulation. It is considered safer and more effective than spraying application. In this study, biopolymer of poly (3-hydroxy-butyrate) or P (3HB) was used as the coating agent [1]. This herbicide was distributed by burying it into the ground at 5-10 cm depth, the active compound would be released slowly in so long term that the growth of weeds would be blocked [2].

Poly (3-hydroxy-butyrate) or P (3HB) is biopolymer earned by bacteria, such as Erwinia sp. USMI-20. From the latest research known that this biopolymer can be used as a coating agent in several pharmaceutical preparations and it won't harm any circumstances [3,4].

Microencapsulates is one of the method used to make slow release pharmaceutical formulation. The principle of this method is the slightly coated of solid particle, liquid drops and liquid dispersion of the coating agent. In this study, metsulfuron-methyl is used as the herbicide model [5].

MATERIALS AND METHODS

Materials and Equipment's
Propeller Mixer (Scientific Stuart), UV-VIS spectrophotometer (Shimadzu), IR Spectrophotometer (Jasco), analytic weighing equipment, photo-microscopic (Optilab Viewer), microscope completed with micrometer, dissolution tester (Hanson Research), and glass equipments, metsulfuron-methyl, P(3HB) (Aldrich chemical), chloroform, HPMC 4000, distillation water and acetonitrile (Sigma).

Raw Materials Examination
The examination of metsulfuron-methyl consisted of organoleptic test, solubility and IR spectra. P(3HB) was produced and characterized based on its physic, chemistry and physical-chemistry characteristic by previous researcher, such as NMR spectrum Identity, GC spectrum, melting point, molecular weight and solubility [5]. The examination of HPMC 4000 based on requirements in United States Pharmacopeia XXX and Handbook of Pharmaceutical Excipients included organoleptic test and solubility.

The Production of metsulfuron-methyl herbicide microcapsule
Microcapsule is produced based on the formula as shown in Table 1.

Table 1. Formula of Microcapsule

Materials	**Formula**			
	F0	*F1*	*F2*	*F3*
Metsulfuron-methyl (mg)	0	500	500	500
P(3HB) (mg)	250	250	500	1000
Chloroform (ml)	10	10	10	10
HPMC 4000 (mg)	350	350	350	350
Distillation Water (ml)	100	100	100	100

P (3HB) was dissolved into 10 ml of chloroform and constantly stirred until homogenously dissolved in Erlenmeyer, while 350 mg of HPMC 4000 was put into a beaker glass containing 100 ml of distillation water and constantly stirred using a propeller (dispersion phase). Then, 500 g of metsulfuron-methyl was added into HPMC solution and stirred until homogenously mixed. After that, P (3HB) solution was poured drop by drop using dropped pipette into the dispersion phase and stirred constantly for about 5 hours under 700 rpm until whole amount of chloroform evaporated. Finally, the forming microcapsules were collected via filtration process on the sieve paper and dried in the drainage case.

Evaluation of metsulfuron-methyl herbicide microcapsules
- **Morphology of microcapsules**

The physical appearances of microcapsules was observed under photomicroscope through a caption at 40 times of expansion.

- **Particle Size Distribution**

Particle Size Distribution obtained was measured by using microscope completed with calibrated micrometer. Microcapsules were suspended into paraffin liquid, then dropped on the object glass and observed under the microscope as 300 particles. The particles were classified based on certain range of size, then the particle size distribution was determined.

- **The determination of process recovery**

Process recovery was determined by comparing an amount of microcapsules obtained (W1) with an amount of microcapsules theoretically (W_0) in percentage.

The Process recovery = $W1/ W_0 \times 100\%$

- **The determination of active compound recovery**

a. The determination of Maximum Wavelength of metsulfuron-methyl
The determination of 10 µg/ml of metsulfuron-methyl solution in distillation water was taken and measured at the interval of 200-400 nanometers using UV-Vis spectrophotometer, then the maximum wavelength examined. The

determination of 10 µg/ml of metsulfuron-methyl solution in acetonitrile was taken and measured at the interval of 200-400 nm using UV-Vis spectrophotometer, then the maximum wavelength examined.

b. The calibration curve of metsulfuron-methyl
One series of metsulfuron-methyl solutions in distillation water were made with the concentrations of 4, 6, 8, 10, 12 µg/ml. Each absorbance was examined in the maximum wavelength.

c. Quantitative Measurement of metsulfuron-methyl in the microcapsules
5 mg of microcapsule was taken, finely grinded, and dissolved into 10 ml of acetonitrile. The amount level of this compound released was measured by using UV-Vis spectrophotometer in the maximum wavelength.

Compound in microcapsules : C1/C0 x 100%

C1 = Amount of this compound in microcapsule
C0 = Amount of this compound theoretically.

d. Analysis of Infra Red Spectrophotometer
Metsulfuron-methyl, empty microcapsule and metsulfuron-methyl microcapsule were distributed in KBr pellet, the spectra was examined by infrared Spectrophotometer.

e. The examination of releasing of metsulfuron-methyl in microcapsule by dissolution tester equipment
The active compound released was measured via a dissolution method. The dissolution tube was filled with 500 ml of distillation water as the dissolution medium under the temperature of 30 ± 0,5^{0}C. An amount of microcapsules which was equal to 100 mg of metsulfuron-methyl was added in dissolution medium with the stirring velocity of 100 rpm. A quantitative amount of 5mL of the solution was taken at 1, 2, 3, 4, 5, 6, 7, and 8 hours and replaced with another 5 ml of distillation water respectively. The absorbances were read three times for each formula using UV-Vis spectrophotometer.

RESULTS AND DISCUSSION

The examinations towards raw materials of metsulfuron-methyl conducted based on the requirements stated in the literature, such as organoleptic, solubility and Infrared spectrum showed in Table 1. P (3HB) was a raw material produced and characterized by previous researcher based on its physic, chemistry and physical-chemistry characteristic consisted of NMR spectrum identity, GC spectrum, melting point, molecular weight and solubility shown in Table 2.

The examination towards raw materials of HPMC 4000 conducted based on the requirements stated in the United State Pharmacopeia XXX and Handbook of Pharmaceutical Excipients consisted of organoleptic, solubility and loss on drying showed in Table 3.

Table 1. Examination result of metsulfuron-methyl.

No	Examination	Requirements	Observation
1	Organoleptic characters		
	- Formation	Crystal/Powder	Crystal /Powder
	- Color	White	White
	- Odor	Esther-like	Esther-Smell
2	Solubility		
	- Water	Quite difficult to soluble in water	Difficult to soluble in water (1:300)
	- Acetonitrile	Soluble	Soluble (1: 38)

Table 2. The examination result of P (3HB)

No	Examination	Requirements	Observation
1	Solubility		
	- Water	Low	Practically insoluble (1:>10.000)
	- Ethanol	Low	Practically insoluble (1:>10.000)
	- Chloroform	High	Soluble (1: 30)

Table 3. The examination result of HPMC 4000

No	Examination	Requirements	Observation
1	Organoleptic - Formation - Color - Odor	 Powder White Odorless	 Powder White Odorless
2	Solubility - Water - Chloroform - Ethanol	 Soluble in the water forming sticky mass Practically insoluble Practically insoluble	 Soluble in the water forming sticky mass (1:23) Practically insoluble (1:> 10.000) Practically insoluble (1:> 10.000)

Results of photomicroscope examination of metsulfuron-methyl powder, empty microcapsule and metsulfuron-methyl microcapsules from each formula were shown in Figure 1-5. Microcapsule obtained was in spheric form with various sizes based on coating agent ratio.

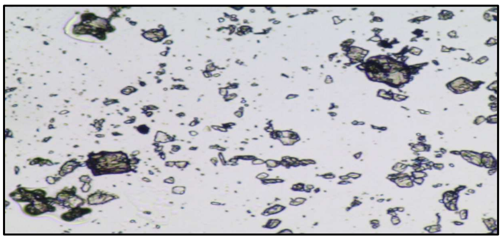

Figure 1. Microscopic form of metsulfuron - methyl powder with 100 times magnification)

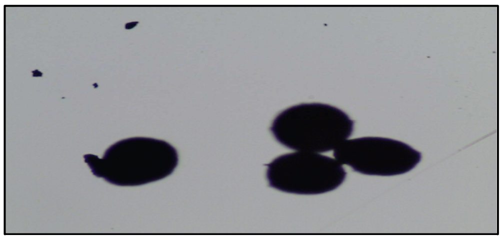

Figure 2. Microscopic form of empty microcapsule with 100 times magnification

Figure 3. Microscopic form of metsulfuron-methyl F1 with 100 times magnification

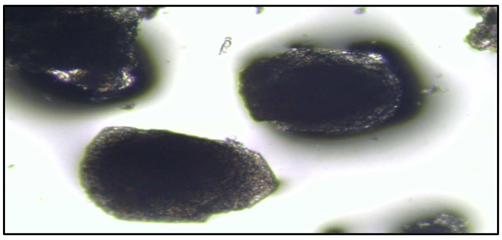

Figure 4. Microscopic form of metsulfuron-methyl F2 with 100 times magnification

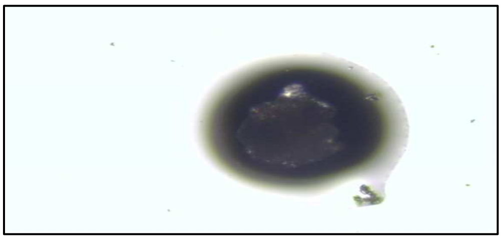

Figure 5. Microscopic form of metsulfuron-methyl microcapsule F3 with 100 times magnification

The weight of metsulfuron-methyl obtained from process recovery and active compound recovery in the microcapsule was showed in Table 4. Besides, the microcapsule size distribution showed result between 1-799.8 μm which is still in the suitable range of microcapsule requirements, showed in Table 5.

Table 4. The weighing of microcapsules earned from this study, the recovery of process and the recovery of the active compound in the microcapsule

Formula	Microcapsule weight obtained (gram)	The recovery of process (%)	The recovery of active compound (%) ± SD
F1	0.6907	92.09	83.56
F2	0.8690	86.90	71.22
F3	1.3750	91.67	67.79
Empty			

Table 5. The evaluation result of particle size distribution

Measurement (μm)	Distribution Frequency of particle size (%)			
	F0	F1	F2	F3
0 – 53.32	36.33	32.33	42	5
53.32 – 106.64	41.33	37	47	49.67
106.64 – 159.96	21.33	19.33	8.67	43
159.96 – 213.28	1	7.33	1.67	0.67
213.28 – 266.6	0	3.33	0.67	0.67
266.6 – 319.92	0	0.33	0	0.33
319.92 – 373.24	0	0.33	0	0.33
373.24 – 426.56	0	0	0	0
426.56 – 479.88	0	0	0	0
479.88 – 533.2	0	0	0	0
533.2 – 586.52	0	0	0	0
586.52 – 639.84	0	0	0	0
639.84 – 693.16	0	0	0	0
693.16 – 746.48	0	0	0	0
746.48 – 799.8	0	0	0	0.33

Note : Formula F1 = Microcapsule with ratio of 1:0.5
Formula F2 = Microcapsule with ratio of 1:1
Formula F3 = Microcapsule with ratio of 1:2

Analysis of Infrared Spectrophotometry

The result of Infrared Spectroscopy analysis of metsulfuron-methyl, empty microcapsule and microcapsule with active compound are shown in Figure 6 and 7.

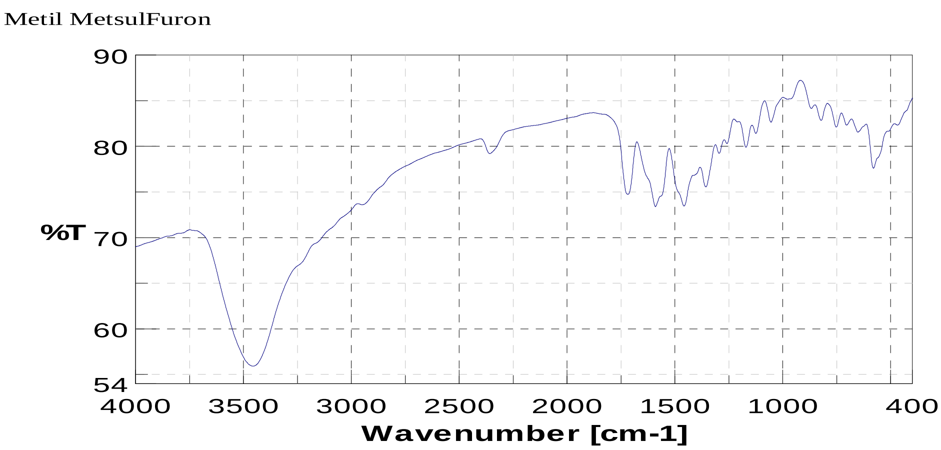

Figure 6: Infrared spectrum of metsulfuron-methyl

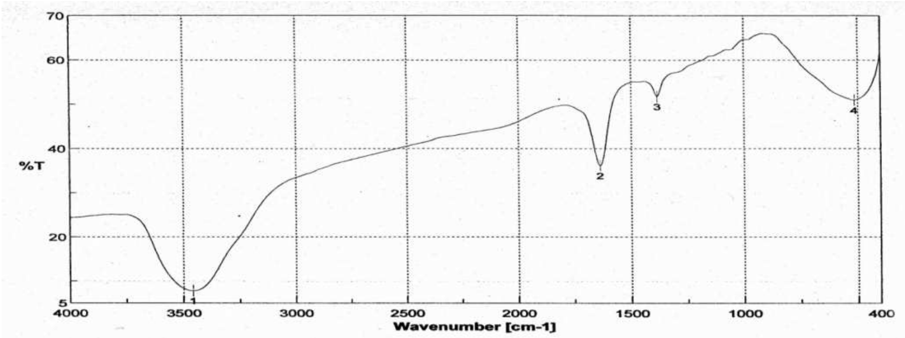

Figure 7: Infrared spectrum of P (3HB)

The releasing test of metsulfuron-methyl microcapsule

The result of releasing test of metsulfuron-methyl microcapsule is shown in Table 7 . The thicker the coating agent, the smaller amount of the active compound released.

Table 7. The result of releasing test of metsulfuron-methyl microcapsule in aquous media

Time (minute)	% The releasing of active compound ±SD		
	F1	F2	F3
60	33,59 ± 0,005	32,44 ± 0,018	15,77 ± 0,053
120	33,76 ± 0,005	33,24 ± 0,022	17,05 ± 0,0007
180	34,48 ± 0,008	34,00 ± 0,0028	21,12 ± 0,022
240	34,84 ± 0,012	34,64 ± 0,042	21,24 ± 0,052
300	35,20 ± 0,020	34,72 ± 0,020	21,32 ± 0,0028
360	38,39 ± 0,008	36,04 ± 0,023	22,67 ± 0,021
420	39,66 ± 0,0028	38,63 ± 0,0028	23,07 ± 0,004
480	41,26 ± 0,0028	39,75 ± 0,009	23,43 ± 0,055

The examination of metsulfuron-methyl was conducted based on requirements stated in the research journal about herbicide consist of organoleptic, solubility and Infrared spectrum identification (Table 1). The result showed that the active compound of metsulfuron-methyl used is suitable to the requirements of European Commission, year of 2000 [7,8].

P(3HB) is a raw material produced and characterized by previous researcher based on its physic, chemistry and physical-chemistry characteristics consist of NMR spectrum identity, GC spectrum, melting point, molecular weight and solubility, and those result are suitable to the requirements.

The examination of HPMC 4000 was conducted based on requirements stated in United States Pharmacopeia XXX and Handbook of Pharmaceutical Excipients consist of organoleptic and solubility, the result showed that HPMC 4000 used is suitable to the requirements in United States Pharmacopeia XXX.

The photomicroscope evaluation showed that microcapsule obtained was in spheric form (Figure 1-5), this form was obtained because the evaporation emulsification happened in the production process of microcapsule. The microcapsule was obtained in form of globules.

Based on Table 4 data, the weighing process of the microcapsules obtained showed that the production of microcapsule using the solvent evaporation emulsification method gave good process recovery result. Microcapsules obtained from formula 1: 0.6907 g, formula 2: 0.8690 g and formula 3: 1.375 g. The recovery process obtained from each formula was: 92.09%; 86.90 %; 91.67%.

The examination of active compound recovery in microcapsules globally showed that the more different amount of P (3HB), the less metsulfuron-methyl found in microcapsule. The most active compound recovery showed in formula 1 : 83.56%, followed by formula 2 : 71.22% and the least formula 3 : 67.79% (Table 4). This showed that the increasing amount of P (3HB) as the coating agent will decrease amount of active compound in microcapsules [9].

The particle size distribution test showed that the particle size of microcapsule stated between 1-799.8 µm (as shown in Table 5). This particle size is suitable to the microcapsule particle size requirements (between 1-5000 µm) based on the method used [5]. The measurement of particle size related to different amount of P (3HB) showed that the more amount of P (3HB), the bigger the particle size. In conclusion, the increasing amount of P (3HB) will change the thickness of microcapsule walls formed.

The result of the microcapsule metsulfuron-methyl released using P (3HB) as the coating agent is shown in Table 7. The release of active compound from microcapsule would be slow down by using P (3HB). After 8 hours dissolution process, amount of active compound from microcapsule dissolved from formula 1 was 41.26%; formula 2 was 39.75% and formula 3 was 23.43 %. It proved that the thicker the coating agent, the slower the active compound released from microcapsule [10,11]. It also showed that P (3HB) can slow down the release of active compound which is expected in sustain release preparations.

CONCLUSION

Three types of the microcapsule formulation was used with the ratio between methyl-metsulfuron, and poly(3-hydroxy-butyrate) of 2:1, 1:1, and 1:2 respectively. Biopolymer of poly(3-hydroxy-butyrate) can be used as the matrix polymer for slow release preparations and influence the release of active substance of metsulfuron-methyl in the objects. The highest value of recovery test of active compound was formula 1 : 83.56%, followed by formula 2 : 71.22% and the last was formula 3 : 67.79%. The process of microencapsulation of metsulfuron-methyl using P (3HB) as coating agent can slow down the release of metsulfuron-methyl. The particle size of microcapsule was stated between 1-799.8 µm in the spheric form.

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REFERENCES

[1] Akmal D, L Fitriani, QA Wangi, PD Asiska, Ismed F, Zaini E, **2015a**, *Res J Pharm, Biol Chem Sci*, **2015**, 1(1):814-822.
[2] Akmal D, Suharti N, Syafrimen Yasin, Maria U., Asiska P. D, **2015b** Journal of Chemical and Pharmaceutical Research 10/**2015**; 2015(79):407-416.
[3] Akmal D, MN Azizan, MIA Majid, *Polym Degrad Stab*, **2003**, 80(3):513-518.
[4]Majid MIA, DH Akmal, LL Few, A Agustien, MS Toh, MR Samian, N Najimudin, MN Azizan, *Int J Biol Macromol*, **1999**, 25(1-3):95-104.
[5] Lachman L, Lieberman HA, Theory and Practice of Industry Pharmacy, 2nd Edition, UI Press. Jakarta. **1994**.
[6] Akmal D, PD Asiska, QA Wangi, H Rivai, A Agustien, **2015c**, *Rasayan Journal of Chemistry*, **2015**, 8(3):389-395.
[7] European Commission, Directorate-General Health & Consumer Protection, *Identity, Physical and Chemical properties metsulfuron-methyl*, **2000.**
[8] FAO specifications and evaluations for plants protections product.metsulfuron-methyl **2001.**
[9] Netty Suharti, Salman, Muslim S, Dwisari D, Febriyenti, Idris, Akmal D, *Res J Pharm, Biol Chem Sci*, **2016,** 7(1): 1691-1698.
[10] Ansel HC, Introduction of Pharmaceutical Preparations 4th Edition UI Press. Jakarta, **2005.**
[11] Muslim S, Salman, Fitriani L, Suharti N, Erizal Z, Febriyenti, Aldi Y, Akmal D, *J Chem Pharm Res*, **2015**, 7(11):478-484.