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Slow release herbicide of 2,4-dichlorophenoxy acetic acid using a biopolymer as matrix of microcapsule

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ABSTRACT

The formulation of microcapsule of 2.4-dichlorophenoxy acetic acid [(2,4-D)] using a biopolymer of polycaprolacton as matrix by solvent evaporation method has been carried out. In the study, three types of the microcapsule formulation was used with the ratio between 2.4-dichlorophenoxy acetic acid, and polycaprolactone, (PCL) 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 polycaprolactone can be used as the matrix polymer for slow release preparations and influence the release of active substance of 2.4-D in the objects. It is also observed that the recovery test of active compound of 2,4-D of formula 2 was of 86.5% w/w. This value was higher than Formula 1 (84.175%w/w) and formula 3 (78.2% w/w). The kinetic model of active compound released from microcapsules with corelation coefficient of near by 1 and followed the zero order kinetic obtained from formula 1.

Keywords: Slow release, microcapsule**,** biopolymer**,** polycaprolactone, 2.4-dichlorophenoxy acetic acid

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INTRODUCTION

Herbicide is one of the globally used pesticides. Herbicide is a chemical substance which has such ability to kill weeds in order to increase the agricultural production. 2.4-D an herbicide which is usually used to manage the weeds. This herbicide selectively inhibits the development of wide leafy weeds. The 2.4-D was usually spread by spraying method and acts systemically once after it has been introduced and translocated into the plants [1].

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. Besides, the distribution of herbicide via sprayer is less effective since it does not reach the root of the plant, heterogenous and short living time. This substance is also has a propensity as an oncogenic agent which possibly cause the liver and kidney damaged and cataracts [2,3].

If the spraying formulation of the herbicide keeps on developed, a serious problem will occur. 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. In the study, polycaprolactone biopolymer was used as a matrix of 2,4-D in order to obtain a slow release formulation. The study was based on the basic concepts of pharmaceutical formulation especially in the field of pharmaceutical agriculture to produce herbicides, pesticides and fertilizers [4,5].

EXPERIMENTAL SECTION

Equipment and Materials

Homogenizer rod (Heidolph RZR 2000®), UV-Vis spectrophotometers (Shimadzu UV-1700 pharmaspec®), Analitical balance (Adam88®), Dissolution tester equipment (Hanson Research SR8 plus®), IR spectrophotometers (Jasco®), Scanning Electron Microscope (SEM) (Jeol®-Japan), polycaprolactone (Aldrich chem.co®), Hydroxy Propil Methyl Cellulose (HPMC4000) (Aldrich Chem.co®), beaker glass, vial, volume pippette, separating funnels, sieve paper, parchment, volumetric pipette, volumetric disk, microcapsules storage, Ocular microscope to measure the particle distribution, Microscope completed with optilab, 2.4-D (Merck), aquadest, chloroform.

Row material Examination

Row material examination performed to 2.4-D included physical examination and dissolution test. The examination performed to polycaprolactone biopolymer was carried out using IR spectrophotometer and dissolution test. The examination of raw material of HPMC 4000 was conducted based on the requirements stated in *United States Pharmacopoeia XXIV* and *Handbook of Pharmaceutical Excipients* included physical examination and solubility test [6,7].

Production of 2.4-D microcapsules formulation

The microcapsule formula of 2,4-D using polycaprolactone as matrix as below:

Table 1. Formula of microcapsule of 2.4-D

Materials	Formula(s)			
	Empty Microcapsules	F1	F2	F3
2.4-D (mg)	0	500	500	500
Polycaprolactone (mg)	250	250	500	1000
HPMC 4000 (mg)	350	350	350	350
Chloroform (mL)	10	10	10	10
Aquadest (mL)	100	100	100	100

Microcapsule production method: an amount of 250 mg of polycaprolactone is dissolved in 10 mL of chloroforms constantly stir until homogenously dissolved in Erlenmeyer. Then, quantitatively count and add as much as 500 mg of 2.4- D. Put an amount of 350 mg of HPMC 4000 into a beaker glass containing 100 ml of aquadest and constantly stir using a propeller. After that, pour both the polycaprolactone and 2.4-dichlorophenoxy acetic acidichlorophenoxy acetic acid solution in the primary glass, drop by drop using dropping pipette and stir constantly for 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 [7].

Evaluation of 2.4-D in Microcapsules Formulation

a. Morphology of microcapsules

Physical appearance of microcapsuled was observed under a photomicroscope through a caption at 40 times of expansion.

b. Particle size distribution

Particle size distribution of microcapsules was determined using a microscope which is completed with calibrated micrometer. Microcapsules were suspended in the aquadest then dropped on object glass and observed under the microscope as 300 particles [8]. The particles were classified in certain range of size and then the particle size distribution was determined.

c. Determination of UV-Vis Spectrophotometry maximum wave lenght of 2.4-D

The UV-Vis spectrophotometry maximum wave length of 2.4-D was conducted by preparing 100 mL aquadest in a volumetric disc containing 10 mg of 2.4-D. The amount of 10 µg/mL was taken and measured at the interval of 200-400 nm using a UV-Vis spectrophotometer equipment.

d. The examination of releasing of 2,4-D active compound in microcapsule by dissolution tester equipment

A calibrative curve was created using the sequences concentration such as 3, 6, 9, 12, 15 µg/mL and followed by maximum absorbance determination. The active compound release was measured via a dissolution method. The dissolution tube was filled with 500 mL of aquadest as the dissolution medium under the temperature of 30 ºC. An amount of microcapsules which was equal to 100 mg of 2.4-D 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 aquadest respectively. The absorbances were read two times for each formula using UV-Vis spectrophotometer [9].

e. Percentage loading of active compound, encapsulation efficiency and percentage yield of microcapsule determination.

The loading of active compound and the encapsulation efficiency were calculated using this following equation :

$$\textbf{\% Loading} = \frac{2{,}4\text{–}D\ mass}{microcapsule\ weight} \text{ x } 100\ \%$$

$$\textbf{Encapsulation Efficiency} = \frac{Amount\ of\ drug\ obtained}{Amount\ of\ drug\ theoritically} \text{x } 100\%$$

Then, the percentage yield of microcapsules obtained from each formulation was calculated using this following equation :

$$\textbf{\% yield} = \frac{Weight\ of\ microcapsules\ obtained}{Total\ weight\ of\ Active\ compound\ -Weight\ of\ estimated\ polymer} \text{ x } 100\%$$

f. Determination of concentration of 2.4-D in the microcapsules

Microcapsules were quantitatively counted for 10 mg, and softly grinded in a (lumpang) before diluted in 10 ml of chloroforms. The solution was separated using a sieve. The concentration of 2.4-D was determined using UV-Vis spectrophotometer at the maximum wavelength of 229.2 nm.

g. Determination of finger print area of 2.4-D using Infra Red spectrophotometer.

An amount of 200 mg of KBr powder was added into 1-2 mg of 2.4-D and gently stir until a homogenous mixture was obtained. The mixture was put into a pressing disk using a mechanic pressing tool. The pressing value was stabilized for several minutes in order to reach an optimum rigidity. Afterwards, the pressed mixture was taken and put carefully on the sample desk of IR spectrophotometer to be analyzed. Another 10 mg of the active compound was put onto the plate and then measured appropriately [10].

h. Microcapsule observation using Scanning Electron Microscope (SEM)

An amount of 10 mg of microcapsule was quantitativelycounted and dried under a vacuum since H_2O free condition was required. The sample then put into the sample holder coverred by Platinum. The sputting protocol was aimed to a less conductive samples. Sample holder was 12-25 mm in long since the wide of contact area would bring any advantages. Double side conductive tape was needed to put the samples. Afterwards, the measurement was carried out[11].

RESULTS AND DISCUSSION

Raw Material Examination

The examination towards raw material of 2.4-D was conducted based on the requirements stated in USEPA (United States Environmental Protection Agency), as shown in Table 2.

Table 2. Raw Material Examination of 2.4-D data

No.	Examination parameters	Requirements	Observation
1	Description - Shape - Colour - Odor	 Powder White Inconvinient odor	 Powder White yellowish Inconvinient odor
2	Solubility - In water - In - In Chloroform	 Slightly soluble Slightly soluble soluble	 Slightly soluble Slightly soluble soluble

The examination toward the PCL fullfilled the requirements as shown in Table 3.

Table 3. Examination of polycaprolactone (PCL)

No	Examination parameters	Requirements	Observation
1.	1. Pemerian - Shape - Colour - Odor	 Pellet White Odorless	 Pellet White Odorless
2.	2. Solubility - Water - Chloroform	 Insoluble Easily dissolved	 Insoluble Easily dissolved

The examination of raw material of HPMC 4000 was conducted based on the requirements stated in *United States Pharmacopoeia XXIV* and *Handbook of Pharmaceutical Excipients* included physical examination and solubility test as shown in Table 4.

Table 4. The examination of raw material of HPMC 4000

Examination parameters	Requirements	Observation
1. Description		
- Shape	Powder	Powder
- Colour	White to cream	Creamy white
- Odor	Odorless	Odorless
2. Solubility		
- In water	Soluble and form the gummy mass	Soluble and form colloids
- In ethanol	Practically insoluble	Practically insoluble
- In Choloform	Practically insoluble	Practically insoluble

Evaluation of Microcapsules

Result of infrared spectrophotometer analysis showed by Figure 4, 5, 6, and 7. Result of the examination of empty microcapsules using Scanning Electron Microscope (SEM) was shown by Figure 7 while toward the 2.4-D shown by Figure 8, 9, 10 and 11. Result of determination of 2.4-D concentration in the micromolecules was shown by Table 7.

Determination of maximum wavelength and creating the calibration curve of 2.4-D

The maximum wavelength of 2.4-D solution was determined at 229.2 nms as shown by Figure 1.

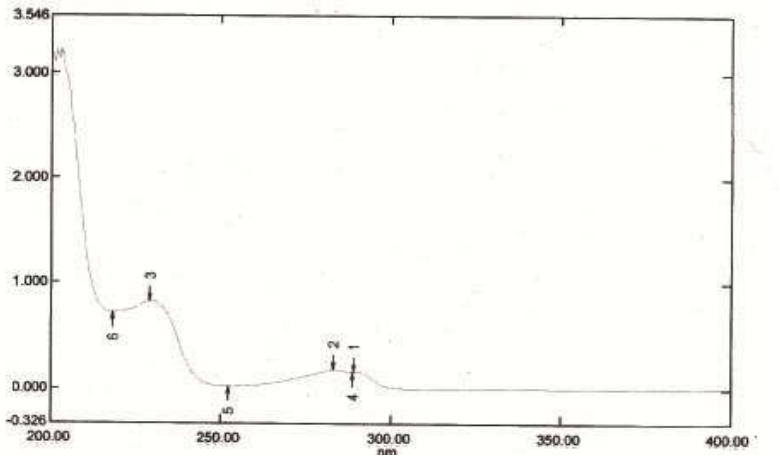


Figure 1. UV spectrum showing the maximum wavelength of 2.4-D

Calibration curve was created using the equation of y= 0,04048x – 0,00555.

Table 5. Absorbances obtained from standart solution of 2.4-D

Concentration µg/mL	Absorbance (A)
0	0,000
3	0,116
6	0,239
9	0,355
12	0,481
15	0,603

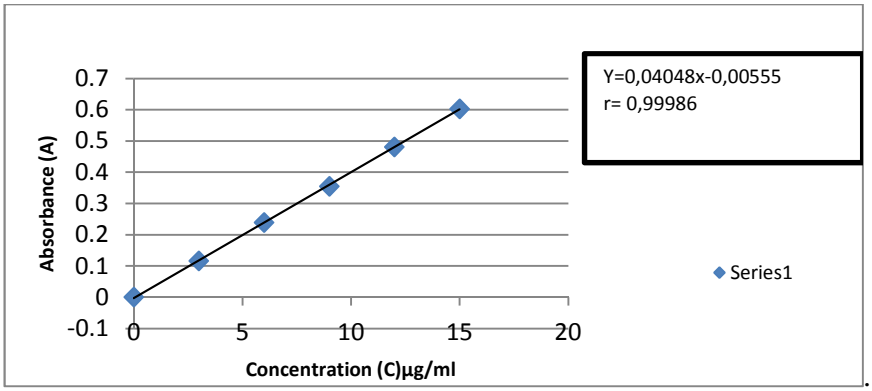


Figure 2. Calibration curve of 2.4-D

Percentage solubility of 2.4-D

The result obtained from solubility test of 2.4-D formula1 (F1) to formula3 (F3) in the form of microcapsules in the medium of water showed that there was decrease of releasing rate of active compound from microcapsules.

Table 6. Percentage of 2.4-D microcapsule (Formula1 to Formula3) diluted in water

Time (Minutes)	F1 (%)	F2 (%)	F3 (%)
60	55,74512	59,91385	34,4383
120	57,5501	61,99443	38,05745
180	58,56372	62,54022	46,08842
240	58,56372	62,69616	49,12928
300	59,49937	62,93007	50,84463
360	59,96719	63,24196	54,19737
420	60,51298	63,70978	54,74316
480	62,46225	64,56746	56,92634

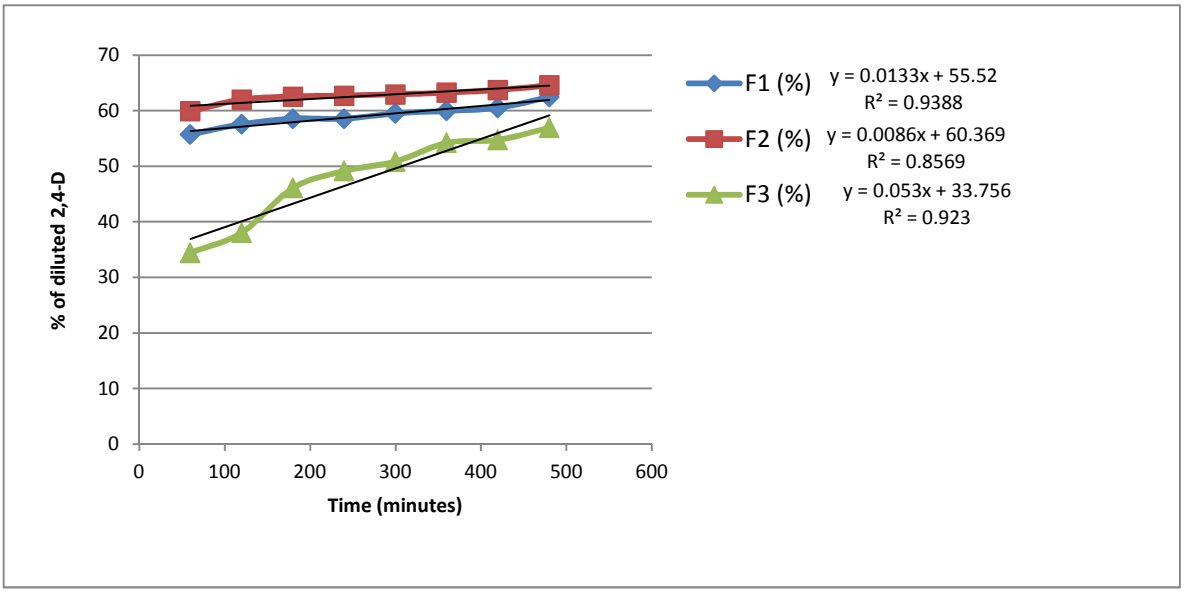


Figure 3. Curve of the percentage solubility of 2.4- D in the form of microcapsules

Result of solubility test showed that the amount of biopolymer used to form the microcapsules affected the amount of active compound released from the microcapsules. Using the zero order equation, Formula 1,2 and 3 yielded the regresion value of 0.968, 0.925. and 0.960 respectively. It meant that formula 1 followed an order 0 kinetic since the regresion value was nearby to 1. On order 1 equation, the regresion value of three formulas were 0.9685, 0.9129, 0.9428. Using Korsemeyer Peppas method, the regresion value of was nearby to 1 with the value was 0.987 while using the Langen Bucher method, the formula 3 also yielded the similar regresion value, 0.9878. The similar result also showed by the Higuci equation [7,9, 12].

Analysis using Fourier Transform Infra Red Spectroscopy (FTIR)

Spectroscopy analysis of active compound, coating and forming microcapsules was conducted using Fourier Transform Infra Red (FTIR) spectrophotometer. The result of 2.4-D showed that there was detected at the wavelength of 1227.46 and 1091.28 (Figure 4.) Carbonyl was redorded at 1300 – 1000 /cm. Concerning the literature review, an aromatic C-C bond was found at 900-690 as shown by the wavelength of 892.67 and 908.54 /cm. Unfortunately, the hidroxyl group which was attach to the main compound was not detected at its estimated wavelength. This might because the active compound was hydrolized due to the inproper storaging.

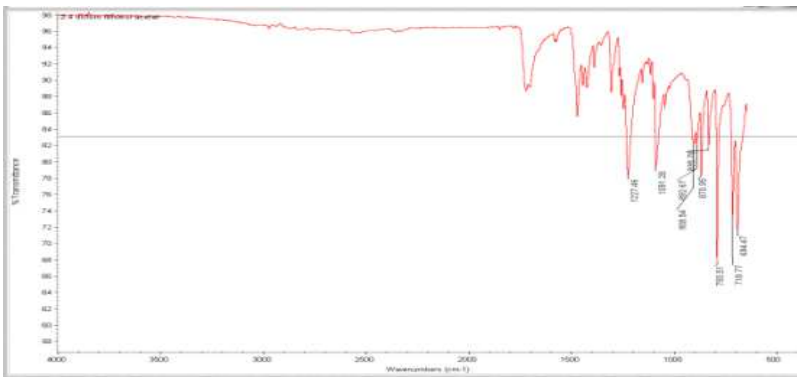


Figure 4. Fourier Transform Infrared (FTIR) spectrum of 2.4-D

FTIR analysis of polycaprolactone was shown by figure 5. Concerning the literature, at the wavelength of 1721,86 cm^{-1}, a carbonyl functional group from a carboxylic acid was detected. While at the wavelength of 1239,02-1175, 38 cm^{-1}, the carbonyl (C=O) of an acethone or an ester was recorded.

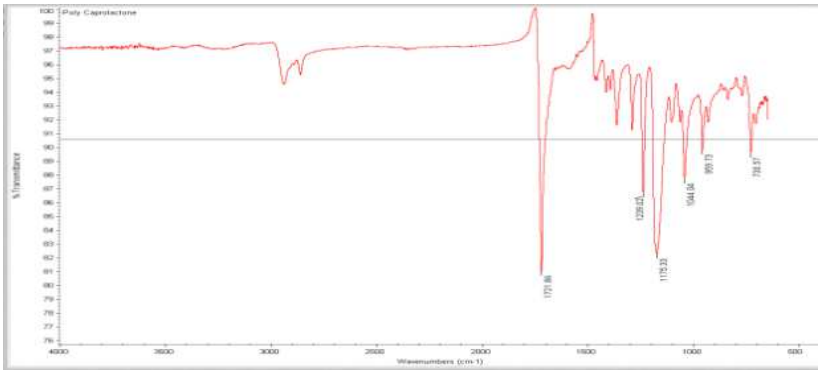


Figure 5. FTIR spectrum of polycaprolactone

The FTIR analysis of empty microcapsule was shown by Figure 6. Based on the figure above, a hydroxyl group was found at the wavelength of 3456,78 cm^{-1}, C=C-H was at 2957,3 cm^{-1}, carbonyl(C=O)at 1725,01 cm^{-1}, C=C at 1638,3 cm^{-1}, and C-H bond was detected at the wavelength of 1471,42 cm^{-1}.

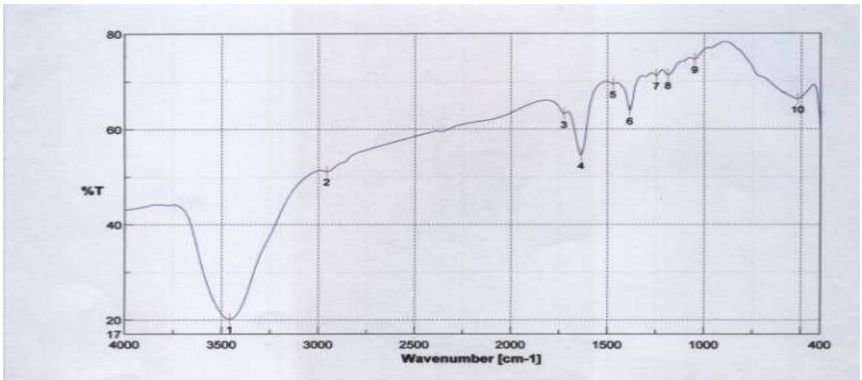


Figure 6. FTIR spectrum of empty microcapsules polycaprolactone

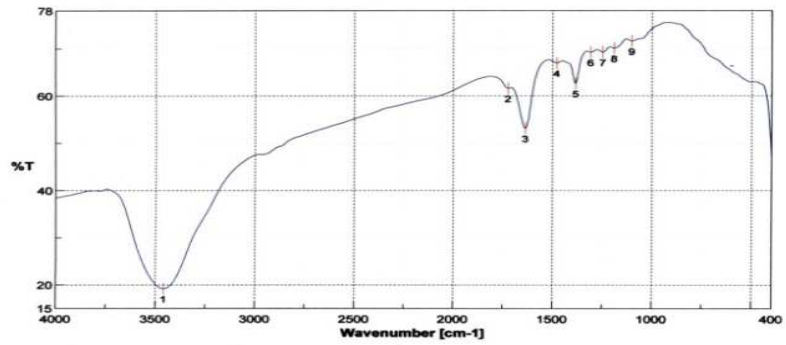


Figure 7. FTIRspectrum of 2.4-D microcapsules coated with polycaprolactone

As shown by the figure 7 above, the spectrum of 2.4-D microcapsule showed that the hydroxyl group was detected at the wavelength of 3458,71 $cm^{-1}$and C=O at 1723,09 cm^{-1}.

Microcapsule of 2.4-D was formed using three formulations with the ratio of 2.4-dichlorophenoxy acetic acidand polycaprolactone were 2:1, 1:1, 1:2 respectively. The method used in the study to form the microcapsules was solvent evaporation emulsifying method. This method was used because it was efficienct and easy to be performed. Polycaprolactone was easily dissolved in the evaporating solvent such as chloroform. The uses of each component of 2.4-dichlorophenoxy acetic acid microcapsules werechloroform as dissolving agent toward `the biopolymer and water as its dispersing phase, HPMC as the emulgator to stabilize the forming emulsion in the microencapsulation process.

The result of microcapsule evaluation using a photomicroscope showed that the form of microcapsule was spheric with various size. The size formed depend upon the amount of the coating agent used. The more coating agent the thicker the microcapsule covered the active compound.

Scanning Electron Microscope (SEM)

Figure 8 below showed that there were pores on the surface of the microcapsules. The empty microcapsules were more compact compare to 2.4-D microcapsules which showed any cracks. This phenomenon was due to the interaction between the biolpolymer and the active compound. The surface of empty microcapsules was flat whereas the surface of 2.4-D microcapsules was uneven. The condition occur because the active compound was covered by the biopolymer.This event promoted the *burst effect*where the active compound lied on the surface of the microcapsule that caused active compound released earlier. This effect cause the order reaction changed into zero [7, 12].

Figure 8. Result of SEM of empty polycaprolactone microcapsules with 1000 times magnification

The weighing of microcapsules formed in the study were: 1. Empty microcapsules with 250 mg of polymer formed 188.3 mg of microcapsules and recovery value of 75.32%; 2. On formula 1 containing 500 mg of active compound and 250 mg polycaprolactone formed 500 mgof microcapsulesand the recovery value of 66.7% whereas the active compound recovery 84.175%; 3. On Formula 2, the amount of 500 mg of 2.4-D and 500 mg of biolpolymerformed 791.9 mg of microcapsules with the recovery of 79.19%, whereas the value of active compound recovery of86,8%; 4. On Formula 3 containing 500 mg of 2.4-D and 1000 mg of polycaprolactone formed 1331 mg of microcapsules with the recovery value of 88.73% whereas the active compound recovery of 78.72%.

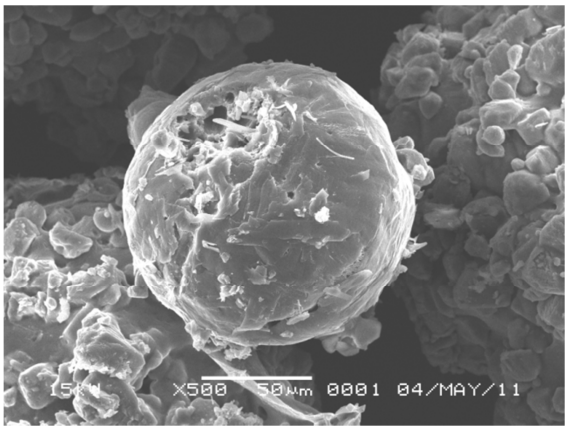


Figure 9. Result of SEM of Formula 1of 2.4-D microcapsule with 500 times magnification

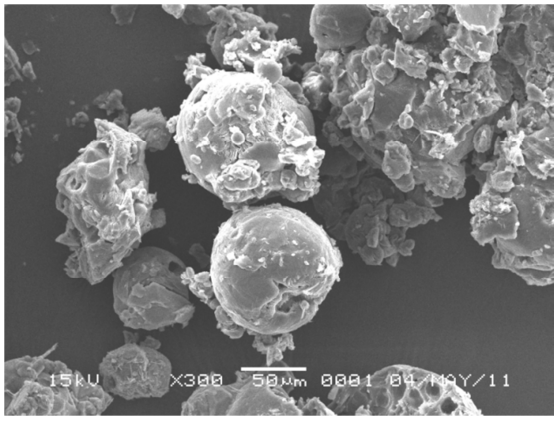


Figure 10.Result of SEM of Formula 2 of 2.4-D microcapsules with 300 times magnification.

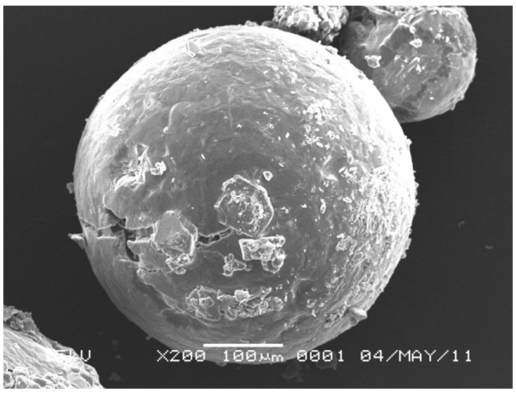


Figure 11. Result of SEM of Formula 3 of 2.4-D microcapsules with 200 times magnification

The recovery value were less than 100%. This occured because an amount of total mass was left behind the container. Besides, the highest of recovery value was obtained from Formula 2 Which contained the same amount of active compound and microcapsules formed (ratio 1:1). Whereas Formula 3 gave the lowest recovery value since the high amount of coating agent used covered the small amount of active compounds.

Table 7. Result of content determination of active compound in microcapsules and content percentage of 2.4-D in the microcapsules

Microcapsules	Weight of micarocapsuled obtained (mg)	Recovery value of microcapsule (%)	Recovery value of active compounds (%)
F0	188.3	75.32	
F1	500	66.7	84.175
F2	791.9	79.19	86.5
F3	1334	88.73	78.72

Explanation :
F0 = 250 mg of polycaprolactone

F1 = 250 mg of polycaprolactone +500 mg of 2.4-dichlorophenoxy acetic acid
F2 = 500 mg of polycaprolactone +500 mg of 2.4-dichlorophenoxy acetic acid
F3 = 1000 mg of polycaprolactone+500 mg of 2.4-dichlorophenoxy acetic acid

Particle size distribution

The microcapsules in the present study formed in the different size. The highest homogenicity value was obtained from formula 2 which the value of 83.33% and the interval of 0-66,665 µm. Furthermore, formula 1 with 73% with the similar interval and formula 3 with the higher scales 79.998 – 133.33 with the value ofµm52%. This showed that the higher amount of polymer, the greater the particle size frequency obtained. The intervals obtained were based upon the literature that using a solid core in solvent evaporation method, the diameter ranged from 5 to 5.000 µm [7,9, 12].

Table 8. Result of particle size distribution

Size range (µm)	Avarage of diameter	Frequency of particle size distribution (%)			
		Formula 0	Formula 1	Formula 2	Formula3
0 - 66.665	33.33	70.67	73	83.33	1
79.998 - 133.33	106.66	20.34	22.33	13.33	52.67
146.663 - 199.995	173.33	3.67	3.67	3	27
213.328 - 266.66	239.99	1.33	0.67	0.33	12
279.993 - 333.325	306.66	1	0.33	0.33	4
346.658 - 399.99	373.32	1.33	0	0	3.67

Explanation:
Calibration :
3 scales of ocular = 4 true scales, 1true scales= 0.01 mm
1 scales of ocular= $\frac{4\ x\ 0.01\ mm}{3}$ = 0.01333 mm = 13.33 µm

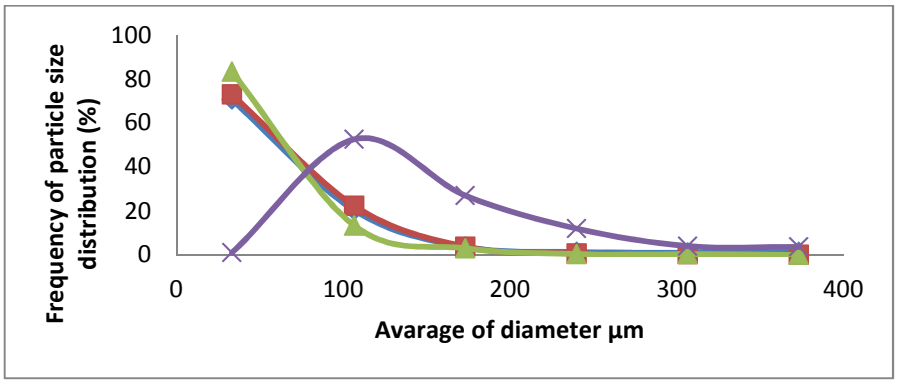
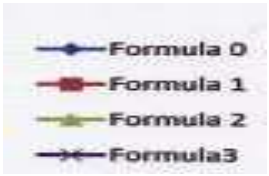


Figure 16. Curve of particle size distribution

Explanation :



CONCLUSION

Our studies showed that polycaprolactone which was used as the coating agent to 2.4-D affected the release of active compound. The recovery value of 2.4-D of formula 2 was of 86.5%. This value was higher than Formula 1 of 84.175% and formula 3 of 78.2%. The kinetic model of active compound released from microcapsules with corelation coefficient of nearby 1 and followed the zero order kinetic obtained from formula 1.

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