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Microencapsulation of Verapamil Hydrochloride using Poly (3-hidroxybutyrate) as Coating Materials by Solvent Evaporation Method.

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ABSTRACT

Formulations of slow released verapamil hydrochloride microcapsules using poly (3-hidroxybutyrate) [P(3HB)] as coating materials have been studied. Microcapsules were prepared by solvent evaporation method using drug-polymer ratio of 1:1, 1:2, and 1:3. Microcapsules obtained were evaluated by infrared spectroscopy, Scanning Electon Microscope (SEM), particle size distribution, percentage of drug loading, dissolution test and drug release kinetic study. *In-vitro* dissolution profile testing was performed using paddle method in phosphate buffer medium at pH 7,4. Infrared spectra showed that there was no chemical interactions between verapamil hydrochloride and P(3HB) during microencapsulation process. SEM testing showed that microcapsules were spheric in shape. Particle size distribution of verapamil hydrochloride microcapsules were in range of 34–340µm, influenced by concentration of P(3HB). The percentage of drug loading for Formula 1, 2 and 3 were of 36.3±0.93; 24.5±0.66 and 20.8±1.62%, respectively. The percentage of dissolution efficiency in formula 1, 2, and 3 were of 26.9±0.03; 25.6±0.27, and 23.6±0.12%, respectively. The release kinetic model of verapamil hydrochloride from microcapsules followed Langenburcher model equation. There were significant differences of efficiency of dissolution among formula (sig <0.05) Incerasing the concentration of P(3HB) could increase the inhibition released of verapamil hydrochloride from the microcapsules. In conclusion, P(3HB) could be used in the microencapsulation formulation of verapamil hydrochloride for sustained drug delivery.

Keywords: Poly (3-hidroxybutyrate), coating, microencapsulation, verapamil, solvent evaporation.

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INTRODUCTION

Slow release dosage forms are designed for extend release of drug gradually into the gastro intestinal to prolong the release and duration of action of the drug. The objective of slow released formulation also to reduce the adverse drug reaction related with fluctuation of drug concentration in blood. The main objective of controlled release preparations is to achieve an extended therapeutic effect and to minimize undesirable side effects caused by fluctuations of drug levels in plasma. Advantages of slow released dosage forms is to minimize the fluctuations of drug levels in the blood so that the effects of pharmacological more stable, reducing the frequency of administration, increase patients satisfaction and compliance, reducing adverse side effects, the patient's condition more appropriately controlled, increasing the bioavailability of some drugs, reducing costs healthcare expenditure due to fewer unit dosage used [1,2].

Microencapsulation is a method for preparation of a relatively thin coating on small particles of solids, liquid droplets, and the dispersion liquid using coating materials. Microcapsules size is between 1-5,000 µm. The uniqueness of microcapsule is the small coated particles that can be implemented for manufacturing various pharmaceutical dosage forms [3,4]. In microencapsulation process several factors such as core state, stability, and the type of coating materials should be considered. One of most popular coating material nowadays is polyhydroxybutyrate or P (3HB). It was reported firstly by Lemoigne, a microbiologist from the Institute Pasteur in Paris in 1925. He discovered that P(3HB) found in bacterial cells of *Bacillus megaterium*. P(3HB) is decompose into acid D(-)-3-hydroxybutyrate, a normal metabolite available in human blood. In addition, P(3HB) also is biocompatible and not toxic to cells (Pal Paul, 2002). Because of its nature, it was suitable to be used as a coating material in the preparation of microcapsules [5].

There are several methods that can be used in the preparation of microcapsules. They are emulsification solvent evaporation method, emulsification solvent evaporation method, and drug dispersed or dissolved in the polymer solution. Polymer solution containing the drug is emulsified in the dispersing phase and allow the solvent to evaporate and then the microcapsules are collected by the washing process, filtration, and drying [1].

Verapamil hydrochloride is an antianginal drug by calcium channel blockers mechanism. Oral absorption of verapamil hydrochloride is 90% but the bioavalability is 20%. When administered in a single dose of the drug the half-life is 3-7 hours. This drug is distributed shortly and ±90% bound to protein. Side effects of the drug are constipation, headache, dizziness, flushing, hypotension, heart failure, etc. Effective oral doses of verapamil hydrochloride is between 120-180 mg per day divided into 3-4 doses [6].

Because of rapid elimination half-life, repeated dose is required to support the success of the treatment of chronic disease needed an effective therapeutic levels constant over time and patients compliance. Controlled release dosage forms is an alternative to maintain levels of continuous drug therapy and improve patient adherence

Based on the reason above, verapamil hydrochloride microcapsules have been prepared using polymer P(3HB) as a coating material for slow released and prolong drug respon.

EXPERIMENTAL

Equipments

Homogenizer *(IKA® RW Digital),* Fourier Transform Infrared (FT-IR) (*Thermo Scientific NICOLENT ISI*), spectrophotometer UV-Vis (UV-*1700 Pharma Spec, Japan*), analytical balance (*Shimadzu AUX 220*), dissolusion tester (*Hanson Research, Italian*), *Scanning Electron Microscopy (Phenomm pro-X, Netherlands*), oven, others glass apparatus.

Methods

Verapamil hydrochloride microcapsules were prepared following 3 formulas in a Table 1 below with ratio of verapamil hydrochloride and P(3HB) 1:1, 1:2 and 1:3.

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Table 1: Formula of microcapsules

Materials	Formula		
	Fl	F2	F3
Verapamil hydrochloride (mg)	500	500	500
P(3HB) (mg)	500	1000	1500
Dichloromethane (mL)	20	20	20
Span 80 (mL)	1	1	1
Liquid Paraffin (mL)	100	100	100

Biolymer P(3HB) was dissolved in dichloromethane in a beaker. Verapamil hydrochloride was added into the biopolymer solution and stirred until dissolved. In another glass beaker, liquid paraffin was mixed with Span 80. P(3HB) solution was added dropwise into the latest solution and emulsified in a homogenizer at a speed of 700 rpm for 5 hours. The microcapsules were collected by decantation, washed with n-hexane until free from paraffin, then dried in an oven at a temperature of 70^{o}C for 30 minutes.

Evaluation of Microcapsules

Spectroscopy of dried microcapsules were analyzed using FT-IR. Particle size distribution of microcapsules obtained were determined using Optilab was mounted on a microscope ocular lens and connected to the laptop, and calibrated. Samples were placed on the slide and attached to the microscope. The particles will be appears on the laptop screen. Approximately 300 particles were counted.

Determination of verapamil hydrochloride content in the microcapsules

Determination of the maximum wavelength of verapamil hydrochloride. Verapamil hydrochloride main standard solution was prepared by dissolving 10 mg of verapamil hydrochloride in 100 mL of methanol to obtain a concentration of 100µg/mL and 12.5 mL pipette stem into 25 mL volumetric flask then add methanol to mark boundaries. Perform the measurement wavelength of maximum absorption with a UV-Vis spectrophotometer at a wavelength of 200-400 nm.

Calibration curve of verapamil hydrochloride were created using standard solution at concentrations of 20, 30, 40, 50, 60 µg/mL. The absorbance of sample solutions were measured using UV-Vis spectrophotometer at wavelength of maximum absorption of verapamil hydrochloride.

Determination of verapamil hydrochloride contains in microcapsules

Verapamil hydrochloride microcapsules were weighed 50 mg and dissolved with methanol in a 50 mL volumetric flask. Five mL filtered solution was diluted in volumetric flask 25mL. The solutions were diluted 3 times. The final solutions were measured by UV spectrophotometer at maximum absorption wave length of verapamil hydrochloride (n=3).

Determination of Drug Loading, Efficiency Encapsulation, and Microcapsules obtained [7].

Percentage of drug loading in the microcapsules obtained could be calculated using the following equation:

Drug Loading (%) = amount verapamil HCl/amount of microcapsules × 100%

Percentage of microcapsules obtained was calculated using the formula:

% Yield = M/Mo x 100%

While M = amount of the microcapsules, Mo = initial amount of verapamil HCl + initial amount of P(3HB).

Entrapment efficiency = The amount of verapamil measured/amount of verapamil added x 100%

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Sample was placed on the sample holder aluminum at thickness of 10 nm. Samples were observed at various magnification, voltage and current at 5 kV, and 12 mA, respectively.

Dissolution Profile

Dissolution profile test of verapamil hydrochloride from microcapsules was performed using using the paddle method. Dissolution medium used was 900 mL phosphate buffer pH 7.4 and the apparatus set up at temperature of 37±0.5°C. A certain amount of microcapsules equivalent to 120 mg of verapamil hydrochloride was added into dissolution flask, stirred at rate of 100 revolutions per minute. Five mL sample solutions were withdrawn at 10, 20, 30, 45, 60, 120, 240 and 360 minutes. To maintain the liquid volume, 5 mL of fresh and same temperature of dissolution medium was added into dissolution flask. The absorption of sample solutions were measured using UV-Vis spectrophotometer at wavelength of maximum absorption (n=3).

RESULTS AND DISCUSSION

FTIR spectroscopy analysis

The FT infrared spectrum of verapamil hydrochloride, P(3HB) and verapamil hydrochloride microcapsules can be seen in Figure 1, 2 and 3. FTIR spectrum of verapamil hydrochloride shows that the functional group C=C appeared at wave number of 1591.36 and 1516.78 cm^{-1}, whereas -CH3 appeared at wave number of 1460.78, 1416.36 and 1324.92 cm^{-1}. COC appeared at wave number of 1238.67, 1216.74, 1180.74 cm, 1141.26 and 1120.80 cm^{-1}. According to FTIR spectrum from the literature, the functional groups C = C, CH3, and COC appears at 1675-1500, 1475-1300, and 1250-1050 cm^{-1} region. It means that verapamil hydrochloride used meets the requirements [8].

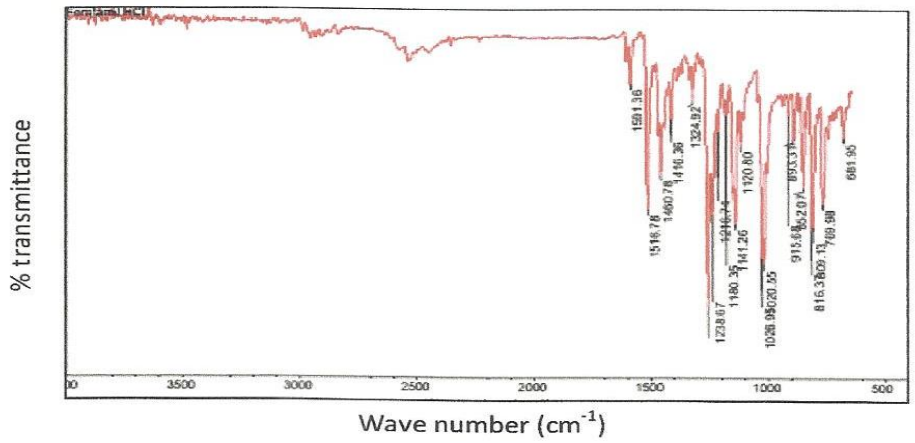

Figure 1: FTIR spectrum of verapamil hydrochloride.

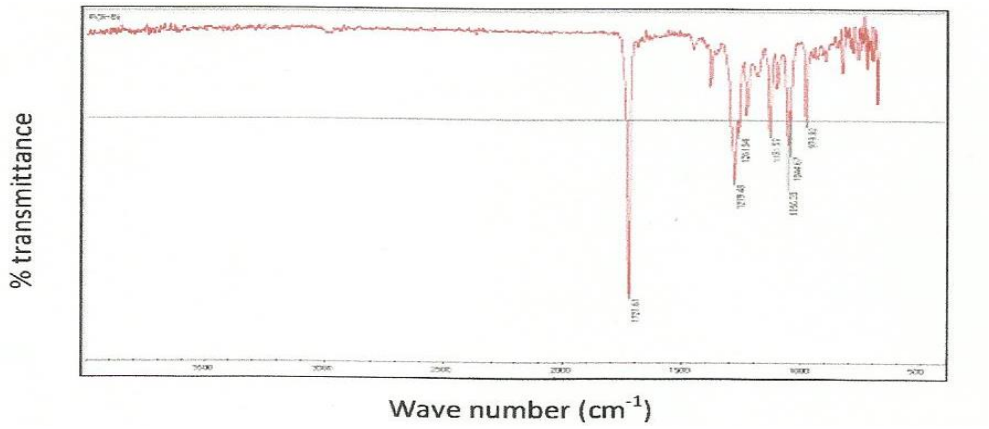

Figure 2. FTIR spectrum of P(3HB).

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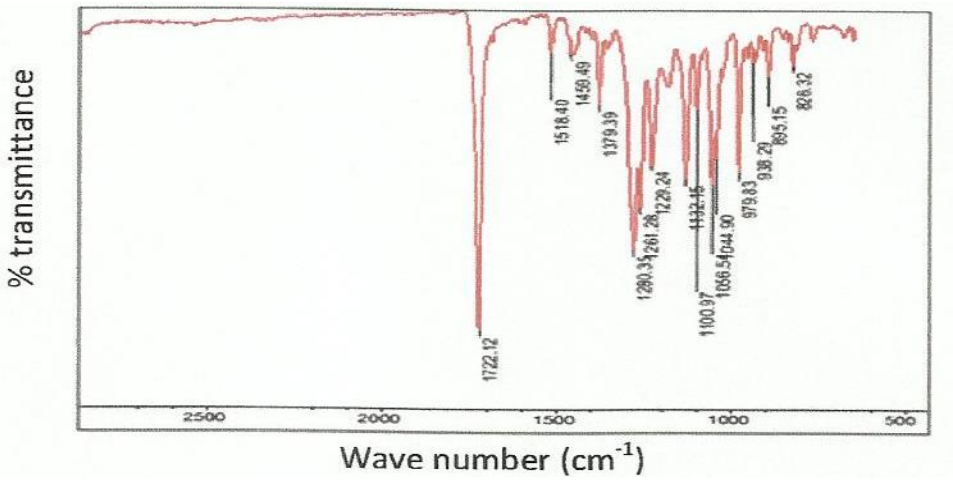

Figure 3: FTIR spectrum of verapamil hydrocloride microcapsules.

FTIR spectrum of biopolymers P(3HB), showed functional group of C=O, C-O, and C=CH appears at wave number of 1721.61, 1056.33, and 979.62 cm^{-1}, respectively. While form literature the group C = O, C-O, C=CH appear at wave number of 1675-1725, 1250-1000, and 1000-650 cm^{-1}, respectively [9].

FTIR spectrum of verapamil hydrochloride microcapsules shown that the valleys depict the functional groups owned by verapamil hydrochloride and the P(3HB) as a coating material. No new group formed. This indicates the absence of chemical interaction between substances in verapamil hydrochloride microcapsules produced.

SEM (Scanning Electron Microscopy)

Microcapsules with and without active ingredient, verapamil hydrochloride, have been evaluated by SEM as shown in Figure 4. From the images of microcapsules without the active substance can be seen that fibers formed at the surface microcapsules prepared without acive substance and no pores detected on that surface. These pores allowed the drug molecules to diffuse out microcapsules. The SEM picture of verapamil hydrochloride microcapsules shown a spherical in shape. However, the microcapsules also looks like aggregates caused by the particle size of the microcapsules were more than 30% in range of 34.1-102µm. It was also supported by the data of the particle size distribution of the microcapsules

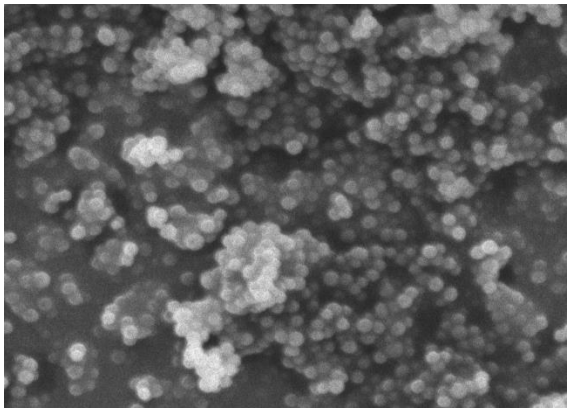

Figure 4: The image of verapamil hydrochloride microcapsules surface detected using SEM at magnification of 2,500 times.

Particles Size Distribution

Particle size distribution was determined using Optilab equipped with micrometer as shown in Figure 5. The microcapsules were observed on 300 particles in each formula at magnification of 10x.

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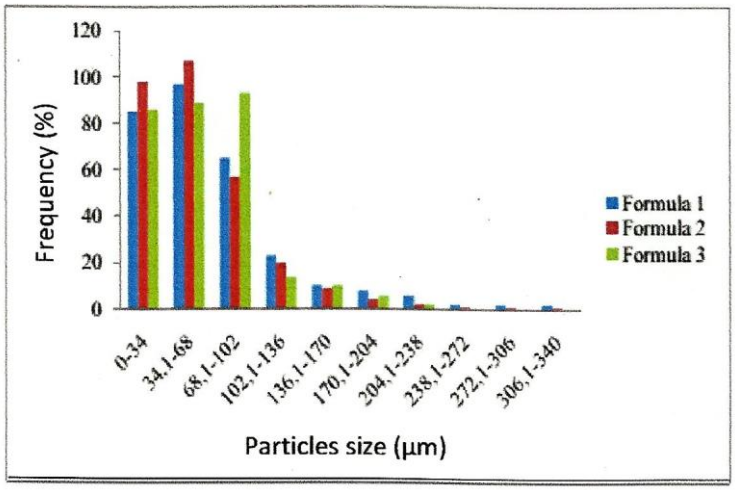

Figure 5: Distribution of particles size of verapamil hidrocloride microcapsules.

Generally, particle size distribution of the microcapsules were at range of 34-340 µm. Most particle size range in Formula 1, and Formula 2 are in the range of 34.1-68 µm. The frequency of Formula 1, and Formula 2 were 32.3, and 35.67%, respectively. 31% microcapsules Formula 3 has a size distribution the largest particles in the range of 68.1-102µm. The size of microcapsule prepared by emulsification solvent evaporation method obtained particle size met the requirements for microcapsules in range of 5-5,000 µm [1]. The higher the amount of P(3HB) used the bigger the particle size of microcapsules. It was assummed that increasing the amount of coating materials caused the thicknening of microcapsules walls [10, 11,12].

Determination of the content of verapamil hydrochloride in microcapsules

The wavelength of maximum absorption of verapamil hydrochloride in methanol was obtained at a wavelength of 279.6 nm. Calibration curve equation obtained was $y = 0{,}010x + 0.116$ ($R^2 = 0.998$).

The amount of verapamil hydrochloride microcapsules obtained in Formula 1, 2, and 3 were 0.79, 1.36, and 2.07, respectively. The amount of microcapsules obtained from Formula 1 and 2 were less than theoriticaly total amount, while in contrary for the Formula 3. The theoretical amount obtained from each formula should be 1, 1.5, and 2 g, respectively. Microcapsules obtained of Formula 1, 2, and 3 were 79.0, 91.1, and 103.8%, respectively. The amount of microcapsules obtained of Formula 1, and Formula 2 showed less than 100%. It may be due to incomplete emulsification process caused in-approproate coating process, and wash out by liquid paraffin [13]. While in the Formula 3 showed the recovery was more than 100%. It may be caused by entrapment of liquid paraffin in the pores of the microcapsules.

Tabel 2: The amount of microcapsules produced and encapsulation efficiency

Microcapsules	The amount of microcapsules produced (g)	Rendement of microcapsules (%)	Drug Loading (%)	Encapsulation Efficiency (%)
F1	0,79	79,0	36.3±0.93	72.7±1,86
F2	1,37	91,1	24.5±0.66	73,4±2,46
F3	2,08	103,8	20.8±1.62	83,2±6,45

The active substance content in the microcapsules obtained

Encapsulation efficiency in Formula 1, 2, and 3 were 72.7±1.86; 73.4±2.46, and 83.2±6.45%, respectively. Verapamil hydrochloride encapsulated were less than 100%. It was caused by inappropriate coating of verapamil hydrochloride during sedimentation process, and carried away by liquid paraffin [13]. In addition, some of verapamil hydrochloride attached on the surface the microcapsules were washed out by n-hexane at washing process.

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The dissolution profile of verapamil hydrochloride from microcapsules using P(3HB) as coating material can be seen in Figure 6. Drug release rate from the microcapsules was highly significantly slower than pure verapamil hydrochloride. It was caused by strongly entrapment of active substance into polymer P(3HB) matrix and drug release from the microcapsules were very slow [14,15].

The active ingredient released rate from Formula 3 was slower than Formula 1 and Formula 2 as well. After 6 hours of dissolution process, the percentage of active substance dissolved from Formula 1, 2, and Formula 3 were 32.5±0.18, 31.6±0.14, and 29.8±0.5, respectively. The higher the amount of P(3HB), the slower the released of verapamil hydrochloride from microcapsules because of the thick walls of the microcapsules. Slow release rate of verapamil hydrochloride from microcapsules caused by hydrophobic characteristics of P(3HB), difficult to dissolve, and hence the penetration of liquid to diffuse limited and slower. Therefore, the time required to release longer.

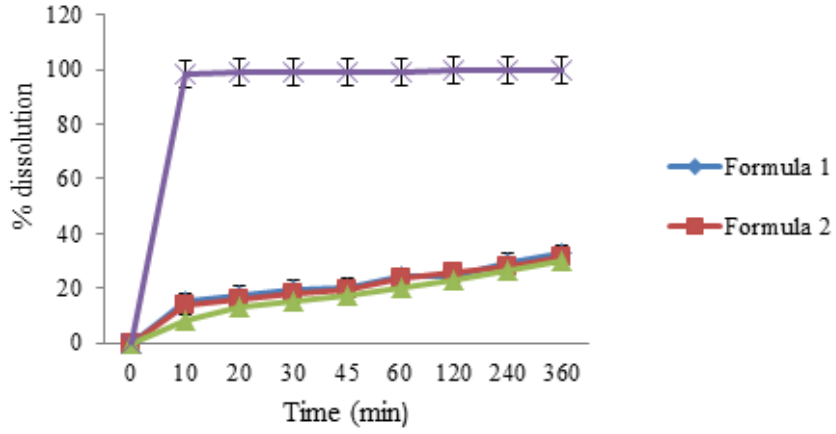

Figure 6: Dissolution profile of verapamil hydrochloride from microcapsules in phosphate buffer pH 7.4 medium

Kinetics model of verapamil hydrochloride released from microcapsules were fixed with following equations; zero-order, first order, Higuchi, Langenbucher and Korsemeyer-Peppas. The kinetics drug released from Formula 1, 2, and Formula 3 followed the Langenbucher equations. It means the release of the drug from the coating and accumulation in the solvent.

The efficiency of dissolution of verapamil hydrochloride from pure sustance, Formula 1, 2, and Formula 3 were anlyzed statistically using one-way ANOVA. Levene's test of homogeneity of variance and signicancy level were 3.391 and 0.103, respectively. It means that the all of variant data were similar, hence ANOVA test using the F test can be done.

ANOVA calculation results show that significant value from ANOVA = 0.000 (<0.05), which means that Ho is rejected shows that the efficiency of dissolution from pure verapamil hydrochloride and all of formula were significantly different. The further Duncan's test showed that dissolution efficiency of verapamil hydrochloride from Formula 1, 2, and Formula 3 were significantly different. From both tests, it can be proved that there were a real difference. The higher the number of P(3HB) in the microcapsules, the slower the release of verapamil hydrochloride from microcapsules.

CONCLUSSIONS

The released of verapamil hydrochloride from microcapsules prepared using ratio of the active substance and P(3HB) 1: 1, 1: 2 and 1: 3 at 360 min were reduced 32.5± 0.18; 31.6±0.14, and 29.8±0.53, respectively. The released kinetics of the active ingredient from the verapamil hydrochloride microcapsules following the Langenburcher model.

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REFERENCES

[1] Benita S. Microencapsulation, Methods and Industrial Applications, New York: Marcel Dekker, Inc. 2006.
[2] Akmal D, R Monica, PD Asiska, S Muslim, ES Ben and Z Erizal. J Chem Pharm Res 2015; 7(7): 558-563.
[3] Muslim S, Salman, Fitriani L, NSuharti, Erizal Z, Febriyenti , Aldi Y, Akmal D.J Chem Pharm Res 2015; 7 (11): 478-484.
[4] Lachman L, Lieberman HA and Schwartz JB (Eds.). Pharmaceutical dosage forms: tablets (Vol. 2). Marcel Dekker, 1990.
[5] Pal S and Paul AK. Current Sci. 2002: 83(12):1565-1568.
[6] Katzung B G, Masters S B and Trevor A J (Eds. Basic & clinical pharmacology, 2004
[7] Khamanga, S M, Parfitt N, Nyamuzhiwa T, Haidula H, and Walker, RB Dissolution Technol 2009; 16(2): 15-22.
[8] Li VH, VHL Lee and JR Robinson. in J.R. Robinson et al. (Eds.), Controlled Drug Delivery Fundamental and Application, 2nd ed., Revised and Expanded, New York: Marcel Dekker Inc. 1987.
[9] Silverstein RM, Webster FX, Kiemle D and Bryce D L. Spectrometric identification of organic compounds. John Wiley & Sons, 2014.
[10] Halder A and Sa B. AAPS Pharm Sci Tech 2006; 46: E1-E7.
[11] Akmal D, PD Asiska, QA Wangi, H Rivai, A Agustien, Rasayan J Chem 2015; 8(3): 389-395.
[12] Akmal D, Afrina D, L Fitriani, PD Asiska, N Suharti, S. Muslim, ES Ben, Z Erizal. J Chem Pharm Res 2015; 7 (8): 683-689.
[13] Dehgan S, Aboofazeli R, Avadi M and Khaksar R. Afr J Pharm Pharmacol 2010; 4(6): 346-354.
[14] Doi Y. Microbial Polyester. New York: UCH Publ. Inc. 1990.
[15] Akmal D, Netty Suharti, S Yasin, Maria U, PD Asiska. J Chem Pharm Res 2015; 7 (9): 407-416