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Use of Eudragit RS PO in the Formulation of Acyclovir Hollow-Microspheres by Solvent Evaporation Technique

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ABSTRACT

This research aims were to formulate a floatable acyclovir hollow-microspheres and control the release of drug using polymer Eudragit RS PO for prolongation of gastric residence time. The floating microspheres were prepared by emulsification solvent evaporation technique. The ratio between drug and polymer in Formula 1, 2, and 3 were 1: 3; 1: 3.5; and 1: 4, respectively. Drug-excipients compatibility were evaluated using Fourier Transform Infra-Red spectrometry, and particle size distributions were characterized by optical microscopes; while shape and surface morphology were observed by scanning electron microscopy. Dissolution test of acyclovir from the microspheres was carried out using paddle method USP Dissolution Apparatus. Density and buoyancy test were performed by using standard procedures. Increasing of polymer concentrations increased the average particle size. The average particle size of Formula 1, 2, and 3 were 26.09, 33.08, and 37.92 µm, respectively. The higher the polymer concentrations, the higher the percentage of drug entrapment. The highest buoyancy and percentage of entrapment observed in Formula 3 were 76.33% for 6 hours and 72.32%, respectively. It was indicated that the Formula 3 has the highest ability to float of. The percentage of drug dissolved for 6 hours from Formula 1, 2, and 3 were 99.95 ± 0.43; 101.54 ± 0.64 and 100.26 % ± 0. 64, respectively. The higher the concentration of the polymer used, the slower the dissolution rate.

Keywords: acyclovir, eudragit RS PO, microspheres, solvent evaporation.

INTRODUCTION

Acyclovir is a synthetic analogue of guanine used in the treatment and prevention of infectious diseases due to herpes simplex virus or varicella zoster. Specific mechanism of acyclovir against herpes viruses interfere with the mechanism of DNA synthesis and inhibit viral replication. The elimination half-lifes of acyclovir are about 2.5-3 hours. Therefore, acyclovir conventional tablet should be consumed 4-5 times a day to achieve an optimal effect [1]. Acyclovir absorption in the gastrointestinal tract is erratic and incomplete [2]. Increasing of acyclovir contact time with absorption site could increase the bioavailability [3]. Therefore, it is necessary to develop a strategy for prolongation of gastric residencec time and controling the release of drug.

A method that can prolong the gastric residence time is a floating system with a small density. It has the ability to swell, then float and stay in the stomach for a longer time. Dosage forms that can be formulated into a floating system is hollow microspheres or also called floating microspheres. One of polymer used in the formulation of hollow microspheres is Eudragit RS PO commonly applied in the preparation of a sustained release dosage form. Eudragit RS PO is a synthetic polymer in form of white powder with a faint amine-like odour. It is soluble in alcohols and acetone, pH independent swelling, non-toxic, and nonirritant. Density of Eudragit RS PO is in range of 0.816 to 0.836 g/cm3. Film formed from this polymer has a low permeability to water [4].

MATERIALS AND METHODS

Equipments

Equipments used are as follows: Fourier Transform Infrared Spectrotometer (Perkin Elmer), Scanning Electron Microscope (HITACHI S-3400N), spectrophotometer (Thermo Scientific), dissolution testing apparatus (SR8 Plus dissolution test Station Hanson Virtual Instrument), light microscope (Zeiss), heating magnetic stirrer (Arec Velp Scientifica), digital scales (Shimadzu-AUX 220), desiccator and other glassware equipment that common used in laboratory.

Materials

Acyclovir, Eudragit RS PO, and Tween 80 were procured from Samparindo, PT. Jebsen & Jessen Chemicals Indonesia, and Brataco, respectively. Acetone, dichloromethane and distilled water were of analytical grade and purchased from commercial sources.

Methods

Preparation of Microspheres

The floating microspheres of acyclovir were prepared by mixing different concentration of polymers. Eudragit RS PO was dissolved in the mixture of acetone and dichloromethane. The polymer solution obtained was added to the dispersion of acyclovir in distilled water and mixed. The mixture above was added dropwise into the solution of 0.5% Tween 80 as an emulsion stabilizer agent, dispersed, and stirred constantly for 1.5 hours using a magnetic stirrer at speed of 300 rpm at 40 °C. The microspheres produced were collected by filtration, washed with distilled water, and then dried at room temperature for 24 hours in a desiccator. The compositions of ingredient of microspheres are shown in Table 1.

Evaluation of Hollow-Microspheres

a. Drug-excipients Compatibility

Drug-excipients compatibility study was performed using a Fourier Transform Infrared (FTIR) spectrometer. The spectra of dried acyclovir microspheres was created in the infrared region consists of a system with the ability to produce monochromatic light in the area of 4000 to 625 cm^{-1}.

b. Particle Size Distribution

Particle size distribution were estimated using a calibrated microscope. A small amount of microspheres was dispersed in liquid paraffin and droped on the slide. Particles size was observed under the microscope and the distribution of particles size were estimated on 1000 particles.

c. Shape and Surface Morphology

Shape and surface morphology of hollow-microspheres were evaluated using a Scanning Electron Miscroscope. Samples were placed on the sample holder aluminum and coated with gold at a thickness of 10 nm. Samples were then observed at a wide range of magnification tool SEM (Jeol, Japan) at voltage and current of 20 kV, and 12 mA, respectively [5, 6].

d. Drug Content

The concentration of acyclovir in microspheres was analyzed using a spectrophotometer. A certain amount of microspheres equivalent to 10 mg of acyclovir was weighed and crushed. The polymer in microsopheres was dissolved using 5 ml dichloromethane and HCl 0.1 N was added gradually. The mixture was filtered into a 50 mL volumetric flask and dilluted with 0.1 N HCl as needed. The absorbance of sample solutions were measured using a UV spectrophotometer at the wavelength of maximum absorption at 255,9 nm. Acyclovir concentrations were calculated using a validated calibration curve.

e. Determination of Drug Loading and Entrapment Efficiency

Drug loading and entrapment efficiency in the microspheres obtained was calculated using the following equation bellow:

$$\% \text{ Drug Loading} = \frac{\text{Calculated amount of acyclovir (mg)}}{\text{Total weight of the hollow microspheres (mg)}} \text{ x } 100$$

$$\% \text{ Entrapment} = \frac{\text{Calclated amount of acyclovir (mg)}}{\text{Theoritical content of acyclovir (mg)}} \text{ x } 100$$

f. Determination the Specific Gravity of the Microspheres.

The specific gravity of the hollow-microspheres were determinated using a pycnometer. Specific gravity of distilled water was first determinated using cleaned and dry pycnometer with a certain volume (a). The empty pycnometer was weighed (b), and then filled with distilled water and weighed again (c). Specific gravity of water was calculated using following equation:

$$\rho \text{ water} = \frac{(c - b)}{a}$$

Once the pycnometer has been cleaned and dried again, it was loaded with one gram of the microcapsules (d). Enough ethanol was then added to the pycnometer to wet the microspheres. The pycnometer was then evacuated and shook to release as much air bubbles as possible. More distilled water was added to the pycnometer untill completely filled, and weighed (e). The specific gravity of microcapsules, ρ microcapsules, was calculated using the equation below:

$$\rho \text{ b} = \frac{(d-b)}{((d-b) + (c-e))} \text{ x } \rho \text{ water}$$

g. Dissolution of Microspheres (USP, 2007)

The *in vitro* dissolution test of acyclovir from the hollow microspheres was carried out using USP type II disSolution apparatus, paddle method, at 37 ± 0.5^{o}C for 6 hr. Dissolution media used were 900 mL of artificial gastric fluid at pH 1.2. A certain amount of microspheres equivalent to 200 mg of acyclovir weighed and dispersed into the dissolution medium. The rate of stirrer was maintained at 50 rpm. At an appropriate interval, 5 mL of samples were withdrawn and replaced with an equivalent volume of fresh and similar temperature of dissolution medium to maintain the constant volume of dissolution medium. Sample solution was diluted as needed and analyzed for the concentration of acyclovir by UV a spectrophotometer at wavelength 254,9 nm. The concentrations of acyclovir released were calculated using the calibration curve obtained.

h. Buoyancy

The buoyancy of the hollow microspheres was carried out by USP type II dissolution test apparatus. 100 mg of the microspheres were spreaded on 900 mL of dissolution medium containing 0.02% Tween 80. The media was stirred at 50 rpm and temperature set at 37 ± 0.5 °C. The floated particles were separated. Sample was then dried on a filter paper and stored in a desiccator for 24 hours. The percentage of floating microspheres were calculated using following equation: [7, 8].

$$\% \, Buoyancy = \frac{\text{Weight of floating microspheres}}{\text{Initial weight of hollow microspheres}} \; x \; 100$$

i. Statistical Data Analysis

The influence of formulation on dissolution efficiency were statistically analyzed using one-way analysis of variance, ANOVA, continued by Tukey's test.

j. Fitting of Kinetic Model

The *in vitro* releases of microspheres were analyzed for establishing kinetics of drug release. The kinetic profiles were fitted with zero order, first order, Higuchi, and Korsemeyer-Peppas models.

RESULTS AND DISCUSSION

Preparation of acyclovir hollow microspheres was performed by emulsification solvent evaporation method with a ratio between drug and polymer as follows 1 : 3, 1 : 3.5, and 1: 4.

As shown in Table 1, the solvent used was a mixture of acetone and dichloromethane using Tween 80 as an emulsion stabilizing agent. Preparation was started with determining the optimization of microencapsulation process including: stirring speed, concentrations of emulsifier, and ratio between solvent and medium. These factors affect the process of microspheres preparation [9,10].

Table 1. Quantity of Raw Materials for Preparation of Microspheres

Ingredients	Formulation Code		
	F1	F2	F3
Acyclovir (g)	2	2	2
Eudragit RS PO (g)	6	7	8
Aceton (mL)	40	40	40
Dichloromethane (mL)	40	40	40
Tween 80 (mL)	1	1	1
Distillated water (mL)	200	200	200

Drug-excipients Compatibility Study

The FTIR spectrometer has been known as an equipment for drug identification. The spectra of the hollow microspheres prepared using different ratio of acyclovir were compared with those of Eudragit RS PO powder, and acyclovir powder. Figure 1, FTIR spectra of acyclovir showed that this drug has several wave numbers. Wave numbers of 3437.50, 3182.09, and 1703.90 cm^{-1} showed the presence of N-H, OH groups, and C = O group, respectively. Wave number of 1624.97 cm^{-1} showed the presence of aromatic groups and alkenes as can be seen in figure 3 [7], Eudragit RSPO FTIR spectrum showed the presence of C-O, C = O, and CH3 group at wave number of 1235, 1730 and 1450 cm^{-1}, respectively.

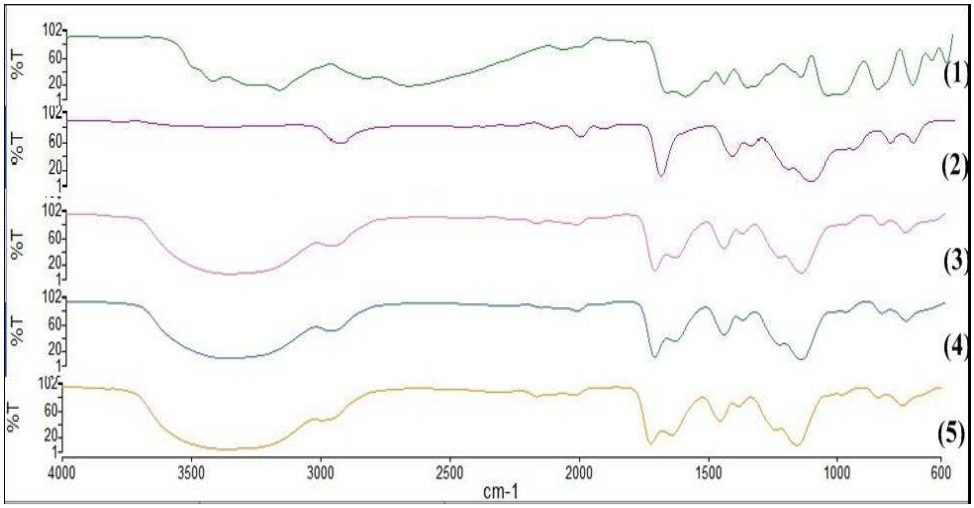


Figure 1. Profile of the FTIR spectra of Acyclovir (1), Eudragit RS PO (2), Formula 1 (F1) (3), Formula 2 (F2) (4), Formula 3 (F3) (5)

FTIR spectra of F1-F3 has an almost similar profile. When It were compared with the IR spectrum of raw materials, the formula has some of the same spectrum with spectrum on Eudragit RS PO. Eudragit RS PO is in wave numbers between 1320-1210, 1700 -1730, and 1450 cm^{-1} indicated the group C = O, CO, and CH, respectively. Acyclovir appears at wave number between 3300-3500, 1480-1690, 650-1450, and 2500-3600 cm^{-1} indicated the group NH, C=N, C=C aromatic, and OH, respectively.

Particle Size Distribution

The particle size distribution of acyclovir microspheres was analyzed using a microscope as shown in Figure 2. An Optilab viewer mounted on the microscope ocular lens. The number of particles calculated was on 1.000 particles with ten times magnification.

The average particle size was increased with increasing the concentration of the polymer. The average particle size of Formula 1, 2 and 3 were 26.09, 33.08 and 37.92 µm, respectively. It may be due to high polymer concentrations resulted in significant increasing in the viscosity of the polymer solution, and reducing stirring efficiency, causing in increasing the particle size [11]. All the formulation obtained meets the requirements of particles size between 1-5.000 µm [9].

Measurement of the amount and size of particles can be estimated using a graph illustrating the relationship between the frequency of the particle size in each formula. From the graph obtained, each formula has a bell-shaped curve depicted a normal distribution curve.

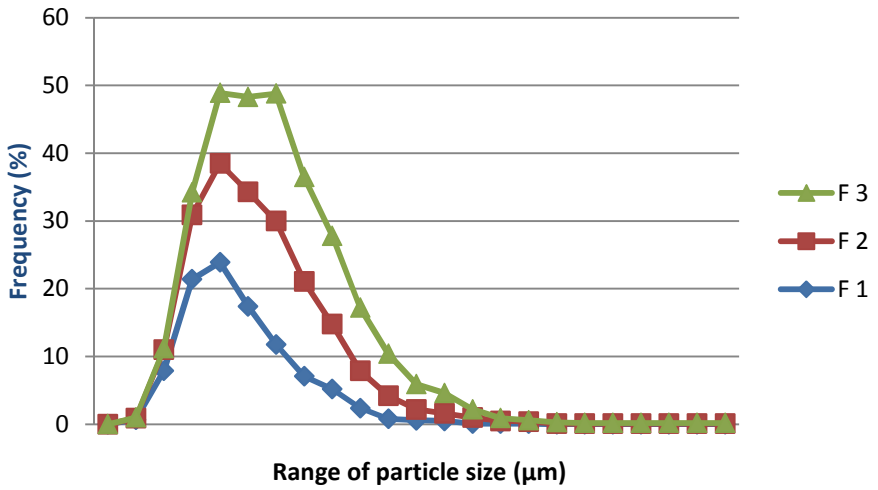
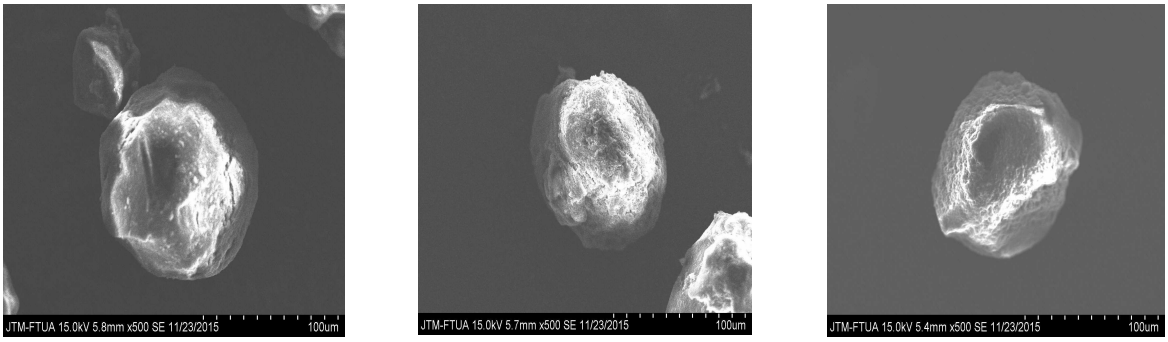


Figure 2. Particles size distributions of microspheres F1 (Formula 1), F2 (Formula 2) and F3 (Formula 3)

Shape and Surface Morphology

The morphology of the active substance acyclovir, Eudragit RS PO and microspheres obtained was observed using SEM (Scanning Electron Microscope). The surface of the microspheres Formula 1 formed uneven due to the process of drying the microspheres to form agglomerates. SEM image of Formulation 2 (Figure 3b) shows that the surface of the microspheres as cratered and uneven due to fast solvent evaporation [8]. SEM photograph of Formulation 3 was similarly with Formulation 2 but craters or pores formed more regular and presence of cavities in the microspheres.



a. F-1 **b. F-2** **c. F-3**

Figure 3. Scanning Electron Microscopy of microspheres F1 (Formula 1), F2 (Formula 2) and F3 (Formula)

Entrapment Efficiency

Acyclovir levels in microspheres were determined using a UV spectrophotometer. The higher the concentration of polymer, the higher drug entrapment efficiency [11]. Entrapment efficiency in Formula 1, 2 and 3 were 64.86, 67.5, and 72.32%, respectively as shown in Table 2. The entrapment efficiency in all formulations was less than 80%. The inefficiency of drug entrapment may be due to dissolve of the active substance during preparation especially at the washing process.

Table 2. Physicochemical Properties and Drug Release Characteristic of Acyclovir Floating Microspheres

Formulations	Entrapment Efficiency (%)	Drug Loading (%)	Buoyancy (%)	Density (g/mL)	Dissolution Efficiency (%)
F1	64,86 ± 0,28	15,42	61,13 ± 2,03	0,9557	68,07 ± 0,25
F2	67,50 ± 0,19	14,66	68,29 ± 0,50	0,9516	65,10 ± 0,42
F3	72,32 ± 0,33	14,39	76,31 ± 0,63	0,9513	59,24 ± 0,29

The buoyancy characteristic of microspheres was estimated using dissolution medium added 0.02% Tween 80. The highest number of floating microspheres was obseved in Formula 3. This is because of the surface of microspheres is porous and the air cavities in it helps the float process. In addition, the larger of average particle size of microspheres also help to maintain its position in the stomach condition.

Buoyancy

The hollow-microspheres were able to float over 6 hours in the dissolution medium 0.1 N HCl. Percentage of buoyancy for Formula 1, 2, and 3 were 61.13, 68.29, and 76.31%, respectively.

The lower specific gravity of the microspheres than 0.1 N HCl (1.004 g/mL) ensured the floating characteristics of microspheres produced [7]. Specific gravity of microspheres Formula 1, 2, and 3 were 0.955; 0.952 and 0.951 g/mL, respectively. Its mean all formulas have a lower specific gravity compare to 0.1 N HCl.

In-vitro Release Kinetics

31% of acyclovir was dissolved from Formula 1 at 30 minutes. It was due to initial burst release of trapped drug on the surface of microspheres. The trapped drug on the surface of microsphere can be seen from the microscopic photo of microspheres. The release of drugs then consistency increase steply until dissolved maximum at the time of 360 minutes. The percentage of drug release from Formula 1, 2, and 3 were 99.5, 101.54, and 100.26%, respectively . As shown in Figure 4, this drug release profiles occurs because of Eudragit RS PO nature release not depend on the pH and have approximately 5% quaternary ammonium group of about for the low permeability to water, so the water is difficult to diffuse and prolong the release [12].

Dissolution time up to 6 hours is not duration enough to control the release of active substances as expected for a controlled release preparation which can reduce the consumption of at least twice the conventional preparations [13]. However, gastric residence time of the floating microspheres more than 6 hours, will help the increasing of drug bioavailability. It was due to the longer contact time could improve the absorption of drug and enables increasing of bioavailability [3]. The swelling time of microspheres obtained was about 3 hours. This swelling time was longer than residence time of conventional tablet in the stomach [14.

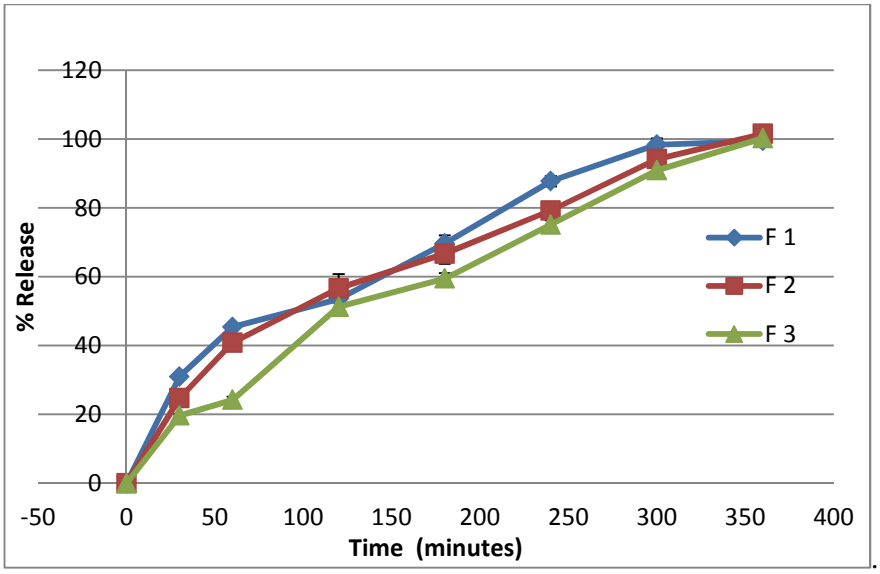


Figure 4. In vitro drug released in artificial gastric fluid

Table 3. The Kinetic Models of Drug Release from the Microspheres

Formulations	Kinetic Models	Linear Regression	Correlation Coefficient (R)
F1	Zero-order	y = 0,215x + 19,48	0.981
	First-order	y = 0,002x + 1,412	0.921
	Higuchi	y = 5,364x + 0,826	0.987
	Korsmeyer-Peppas	y = 0,692x + 0,229	0.988
F2	Zero-order	y = 0,224x + 24,99	0.988
	First-order	y = 0,01x + 1,594	0.940
	Higuchi	y = 5,576x - 4,929	0.996
	Korsmeyer-Peppas	y = 0,479x + 0,779	0.989
F3	Zero-order	y = 0,250x + 14,01	0.991
	First-order	y = 0,001x + 1,543	0.918
	Higuchi	y = 6,192 x -18,91	0.992
	Korsmeyer-Peppas	y = 0,55x + 0,601	0.995

The release data obtained were fitted into various release kinetic models and the results is listed in Table 3. From the correlation coefficient values, it appears that acyclovir release mechanism from Formula 1, and 3 was mainly following Korsmeyer-Peppas equation since it had a higher correlation coefficient value 0.988, and 0.995, respectively. While, the kinetic models of the drug from Formula 2 followed Higuchi kinetics (R = 0.996). Higuchi equation indicates that the release mechanism was controlled by the diffusion of acyclovir from microspheres.

Dissolution efficiency of acyclovir microspheres from Formula 1, 2 and 3 were 68.07, 65.10, and 59.23%, respectively. These results suggested that the increasing concentration of polymers would decreased the rate of dissolution of acyclovir. Dissolution efficiency were statistically different among formulas analyzed by ANOVA ($p<0.05$). More over Tukey's test conducted on the independent effect of each factors. Tukey test results showed that the dissolution efficiency of Formula 1 was significantly different from the Formula 2 and 3.

CONCLUSION

Microspheres obtained is a spherical in shape with uneven surfaces, porous and can float. The highest entrapment efficiency and buoyancy were obtained in the Formula 3 i.e 72.32, and 76.31%, respectively. Eudragit RS PO in the manufacture of microspheres acyclovir can control the release of active substances for 6 hours, while the percentage of dissolved substances maximum of 6 hours for three formulas, F1, F2, and F3 were 99.95, 101.54, and 100.26%. Based on the model of the release kinetics of the active ingredient, microspheres Formula 1 and 3 following the Korsmeyer-Peppas kinetic model (R = 0.988, and 0.995, respectively), while Formula 2 followed Higuchi kinetics (R = 0.996).

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