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Research Paper

The Enhancement Solubility and Stability of Erythromycin Formatted in Solid Lipid Nanoparticles by Utilizing PVA as Stabilizer

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Abstract
Infectious diseases have changed the world order today where the infection is the main cause of illness and death in the world. Erythromycin is a macrolide antibiotic that can generally inhibit the growth of *Staphylococcus aureus* and resistant strains. The free molecule of erythromycin in the part of the body does not reach *Staphylococcus aureus* because it is degraded by the first-pass effect. Nanoparticles can minimize damage to active substances due to first-pass effects because the particles have been protected by biopolymers leading to the minimizing damage of active substances. Formulation of nanoparticles loading erythromycin was used with the following variations in the amount of erythromycin 25 to 100 mg. Erythromycin was formulated by the coated polymer to changes the physics of the erythromycin into a particle. Preparation of erythromycin into nanoparticles was utilized stearic acid polymer, PEG-400, and polyvinyl alcohol using hot homogenization and ultrasonication method. Results showed that the optimum formula was the second formula (F2) with a percentage of encapsulation efficiency of 80.89773±0.11364. The results of the characterization of submicron particle formation such as morphology, diameter (particle size) and distribution (PDI) of F2 were spherical 518.6 nm; 0.096 PDI; and a zeta potential value of -12 mV respectively. The particles loading erythromycin were successfully increasing the stability of erythromycin for up to 5 cycles in terms of the heating-cooling-cycles test and also the solubility in SIF.

Keywords
Lipid-Nanoparticles, PVA, Stearic-Acid, Erythromycin, Stability, Solubility

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1. INTRODUCTION

Bacterial infection is a disease caused by the presence of pathogenic bacteria (Chen et al., 2021; Can et al., 2015; Maglangit et al., 2021). Before this pandemic period, infectious diseases were the main cause of high morbidity and mortality in developing countries but now it is occurring in all countries. As the impact is the increasing use of anti-infective drugs (Vabre et al., 2020; Wong et al., 2020; Blumenberg et al., 2020).

Erythromycin belongs to the class of antibiotic drugs that inhibit the process of protein synthesis by bacteria (Basu and Smith, 2021; Martingano et al., 2020). Erythromycin base (Figure 1) is unstable when it enters the gastric fluid, so it is used in the form of esters or film-coated on erythromycin base and causes a decrease in solubility. In addition, the oral therapeutic dose of erythromycin is quite large, approximately 250 to 500 mg (Özbek et al., 2021; Fateme et al., 2020; Cao et al., 2019).

Previously it was known that lipid particles such as stearic acid can encapsulate drug substances that have low solubility in water (Khongkaew and Chaemsawang, 2021; Mardiyanto et al., 2021). This type of drug substance represents more than 70% of newly discovered drug substances and they are ready to be made into medicinal preparations. Lipid particles are also often chosen because when given orally, the drug that they load will be stable and do not pass through the first-pass effect (Fonseca-Santos et al., 2020; Landh et al., 2020; Wang et al., 2021).

Polyvinyl alcohol (PVA) is used as the stabilizer of the submicron particle formation. PVA is a substance that has high tensile strength and flexibility and functions as a lowering of the surface tension between the 2 phases. The polar hydroxyl group of PVA will bind to water molecules, while the vinyl chain has capability to bind the non-polar molecules so that the emulsion becomes stable (Qiao et al., 2022; Ikeuchi-Takahashi et al., 2016).

Solid Lipid Nanoparticle has been developed by using liquid lipid (Mardiyanto et al., 2021) and also encapsulated antibiotic such as azithromycin to increase the solubility (Bhat-

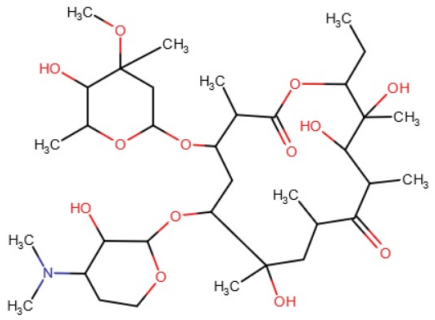

Figure 1. Molecular Structure of Erythromycin

tacharyya and Reddy, 2019) but utilizing PVA as stabilizer of lipid particles loading antibiotics has not been investigated yet. Based on this information, it is necessary to investigate the preparation and characterization of lipid particles loading erythromycin using PVA as stabilizer. Determination of the optimal formula based on the calculation of the percent encapsulation efficiency (%EE). The optimum formula was then evaluated to determine the character of the resulting particle, which included the physical properties using PSA followed by stability and solubility evaluation.

2. EXPERIMENTAL SECTION

2.1 Materials

The active substance in an analytical grade of base-erythromycin was obtained from Sigma-Aldrich®. The materials in synthetic grade are tween 80 (Merc-KGaA®) and PEG 400 (Merc-KGaA®). The solvents in analytical grades are ethanol 96% (Merc-KGaA®), ethyl-acetate (Merc-KGaA®), and aquabidest (OTSU-WFI®).

2.2 Laboratory Work

2.2.1 Preparation of Lipid and Water Phase

The stearic acid was weighed according to the formula and melted in a water-bath controlled temperature (Memmert®) at 60°C. Furthermore, the powder of the drug ingredient erythromycin was weighed and added to the melted lipid using a magnetic stirrer (IKA® C-MAG). In the aqueous phase, there are tween-80 and PEG-400. The weighing of tween 80 and PEG-400 was adjusted relating to the F1, F2, and F3. Then mixed polysorbat-80 and polyethylenglycol-400. The mixing process using stirrer at a speed of 750 rpm (60 minutes) at 60°C. Then this mixtures were stored in a container at 60°C (Rupenagunta et al., 2011; Fu et al., 2014; Chantaburanan et al., 2017).

2.2.2 Formula

The formulas used in this study were three formulas and variations were made on the amount of erythromycin. The amount of stearic acid is in the range of 1 to 2.5%. The amount of tween 80 was used in 2 to 7.5% (Fonseca-Santos et al., 2020; Fonseca-Santos et al., 2020; Chantaburanan et al., 2017). The formula was represented in Table 1.

2.2.3 Formation of Lipid Particles Loaded Erythromycin

Solid Lipid Nanoparticles were formatted using hot homogenization and ultrasonication methods. The manufacture of nanoparticles was begun with the manufacture of the lipid phase. Erythromycin was dispersed into stearic acid which has been heated over a water bath at a temperature of 75°C. Then the water-mixtures were made by adding polysorbat-80, polyethylenglycol-400, and PVA and then stirred (150 rpm) for 3 hours at a temperature of 75°C. The lipid phase was then dispersed into the aqueous phase by drop by drop on a magnetic stirrer for 3 minutes to form an oil-in-water emulsion, and then further sonicated using an Elmasonic® S180H (bath sonicator) for 5 minutes, with an amplitude of 35% to form a nanoparticles. The hot nano-emulsion was quickly poured into 100 mL of cold WFI to obtain nanoparticles. Control SLNs were prepared in the same way without adding erythromycin (Bhattacharyya and Reddy, 2019; Fonseca-Santos et al., 2020).

2.2.4 Purification and Determination of Indirect Encapsulation

A 10 mL particles sample was centrifuged at 12,000 rpm for 30 minutes using Hettich® EBA-BS centrifuge to obtain 2 phases, namely the adsorbed phase and the non-adsorbed phase. Perform non-absorbed phase separation, then add WFI ad 10 mL into the adsorbed phase, and centrifugation was carried out again. This treatment was carried out three times to obtain a solution of erythromycin particles with a non-adsorbed phase. Determination of indirect encapsulation (%EE) was calculated by making a calibration curve using serial amount of erythromycin stock solution with a concentration of 1000 ppm. Measurements using UV-1700 Shimadzu® were adjusted for λ 207 nm. The A results of each measurement are used in the linear regression (y = a + bx) to determine the amount erythromycin as indirect-method.

2.2.5 Measurement of Product Acidity

Measurement of product acidity was conducted by entering the probe of instrument (Luthron pH Electrode®) into the lipid particles loading erythromycin dispersion of F1, F2, and F3 simulant after preparation. After that, the results of pH were evaluated by looking at the input listed on the pH meter screen. Measurements were carried out three times (Mardiyanto et al., 2021).

2.2.6 Characterization of Physical Properties of Particles

Method applied to recognize and evaluate the size of particles, distribution and zeta-potential was PSA SZ-nano by Horiba®. Determination of the mean diameter, PDI, and ζ using the dynamic-light-scattering. The SLN sample was diluted and taken as much as 50 μL of three replication, then put into

Table 1. The Formula of Erythromycin in Lipid Particles

Materials	Function	Amount (g) F1	F2	F3
Erythromycin	Active-substance	0.025	0.050	0.100
Stearic-acid	Lipid	2.5	2.5	5.0
Polysorbate-80	Surfactant	5	5	5
Coglicol-400	Co-surfactant	2.5	2.5	2.5
PVA	Stabilizer	0.2	0.2	0.2
Water-for-injection	Solvent	100 mL	100 mL	100 mL

the PSA cuvette. Particle diameter and ζ measurements were carried out with scattering of two angles (90° to 173°). The visualization of SLN was captured on room temperature without water using electron microscope by Carl Zeiss®. The serial dilution was impacted to the physical SLN though the dispersion of might be added the water up to 1:100 (Mardiyanto et al., 2021).

2.2.7 Thermodynamic and Mechanic Test of Stability

A thermodynamic test was conducted by the Heating-cooling process. The evaluation was followed out by keeping the NLP preparation at the chill temperature for over-night in the refrigerator (Toshiba®). The suspensions were displaced into an oven at warm temperature (40°C) overnight (1 cycle), then the test was continued for six cycles then was observed of phase changing of SLN product as organoleptically also pH-evaluation during the cycles (Almanassra et al., 2021; Freitas and Müller, 1999). Mechanical Test (Centrifugation) SLN samples were centrifuged at 12,000 xg for an half hour of three cycles. The procedures were the same as the step-work of the presence of sedimentation during the year of storage of the preparation. Observations were made by looking at whether or not the separation occurred per 1 cycle (Mardiyanto et al., 2021).

2.2.8 Solubility Test

The purpose of the solubility test was to determine the solubility of the SLN erythromycin formula compare to the base-erythromycin. The solubility test used several types of solvents in the form of distilled water, SIF, sodium hydroxide, sodium bicarbonate solution, acid chloride solution, and SGF of 2 mL each tube. The testing procedure carried out refers to the research conducted by Mahmood et al. (2020). Parameters observed were organoleptic and physical changes of the preparation.

3. RESULTS AND DISCUSSION

3.1 Formation of Nanoparticle Loading Erythromycin

SLN was formatted using hot homogenization method with ultrasonication technique. The sonication method is a dispersing process, which is applied for the formation of SLN-dispersions. This sonication technique is based on the cavitation process. The oil phase was prepared by adding erythromycin to stearic acid which had been melted beforehand in a water bath at 75°C. After that the water-mixtures were made by adding polysorbate-80, polyethylenglycol, and PVA at the same temperature of 75°C (Ikeuchi-Takahashi et al., 2016; Fonseca-Santos et al., 2020; Chantaburanan et al., 2017; Qiao et al., 2022; Khongkaew and Chaemsawang, 2021). The merging of the two phases was carried out by dispersing the lipid phase into the aqueous phase by slowly adding and continuing by agitation process for 3 minutes to form an oil-in-water emulsion and to get the spherical and small-PDI particles and inhibit the aggregation during preparation. The agitation process using a magnetic stirrer can also hinder the coalescence of the 2 phases. Furthermore, after a mixture of the two phases (pre-emulsion) is formed, the solvent was added to Aqua Pro Injection (WFI) up to 100 mL. The choice of WFI solvent is because WFI has a high purity compared to aquadest solvents to prevent contamination and maintain the stability of the preparation. WFI solvents are also free of pyrogens (disease-causing microorganisms). The preparation that has been formed is then homogenized using a bath-sonicator. The principle of this sonication is that the preparation does not directly come into contact with the tool, but through an intermediate medium in the form of a liquid. Ultrasonic waves pass through the liquid in the bath and pass through where the sample is located and can also reduce physical barriers to increase the dispersion ability between the dispersed substance and the dispersion. Sonication is also a standard procedure to prevent agglomeration of samples. Duration of homogenization was impacted to diameter of SLN and tiny diameter is influencing to the constant diameter without surface interaction of grouping particles. Hight-energy of sonic-waves in this method can hinder the coalescence particles and produce the dispersion by the addition of stabilizers (Rupenagunta et al., 2011; Sarathchandiran, 2012; Fu et al., 2014; Mahmood et al., 2020).

3.2 Determination of %Efficiency of Encapsulation (%EE)

Percentage analysis of %EE Solid Lipid Nanoparticles loading erythromycin was carried out by determining the amount of in-direct encapsulated erythromycin. The highest %loading, the most loading substance. Result showed that the F2 had the higher %EE (Table 2). Determination of the %EE starting with a separation process using the centrifuge (12,000 xg for an

half hour). Preparations that have been centrifuged will form 2 phases, namely the adsorbed part and the remaining part. The adsorbed part is amount of substance/drug that is loaded in the preparation as white sedimentation. The non-adsorbed part is the amount of substance/drug that is not loaded, usually a clear liquid that has separated. Upper part has been used and analyzed by a UV-Vis spect to calculate the amount of the unloaded drug. Co-blockpolymer is a non-ionic type of stabilizer that can minimize the surface interaction (Mardiyanto et al., 2021; Kurniawan and Audita, 2021) for facilitating the wetting process of surface of erythromycin, therefor could enhance the %EE. According to amount of stearic acid in F2, stabilizer support with the simultaneous formation of oil/water globule and speed the spread of form in water media and also have naturally amphiphilic abilities and can interact to relatively high amounts of non-soluble drug components. Polysorbate-80 can perform a strength layer around the particle surface, so that it can be inhibited the agglomeration and enhanced the %EE (Figure 2 illustrated the structure of particles). The used of polysorbate-80 to the non-soluble drug can enhance the solubility of drug. This is according to a reduction in the interfacial tension thereby enhance the drug particles. The change of the drug's surface leads to the solubility effect. The using of stabilizer at the certain amount can also reduce the contact of angles between drug and water. On the other hand, after reaching the critical micelle concentration, the amount of surfactant adsorbed decreased with increasing surfactant concentration. The amount of polysorbate-80 can cause the formation of aggregates. SLN have been produced and stabilized by polysorbate-80 but also could be broken or be damaged because the process is influenced by the aggregation so that it can decrease the efficiency of encapsulation. The results of the %EE for each formula were then analyzed by statistical data analysis using SPSS® 24 that F2 was significant difference to F1 and F3 at $p<0.5$.

Table 2. Results of %EE SLN Erythromycin

Formula	Erythromycin (g)	Average %EE±SD	CV (%)
F1	0.025	75.178±0.131	0.153
F2	0.050	80.898±0.114	0.223
F3	0.100	26.201±0.174	0.663

3.3 Physical Properties of Lipid Particles Loading Erythromycin

The choosing formula (F2) for Solid Lipid NPs was further characterized and evaluated. Characterization was carried out to ensure that the preparations made were relevant to the desired character of the requirements. Characterization of the choosing formula for Solid Lipid Nanoparticle loading erythromycin was included in the visualization of particles morphology and measurement of average diameter, PDI, and ζ. The principle of the technique of PSA is used light scattering as dynamically by a light scatter. Then the light was refracted

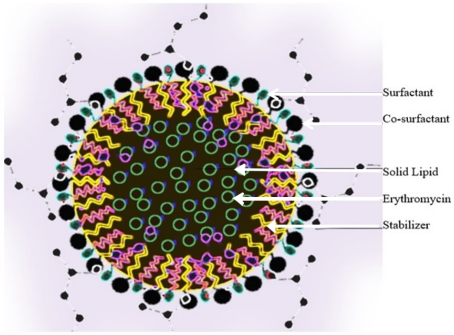

Figure 2. Illustration of Lipid Particles Loading Erythromycin

at several angles while in Horiba SZ-nano is linked to the two main detectors (<90° and >90°) for producing diameter and PDI data also ζ. The tiny particle size is related to increasing the surface area and solubility rate and finally enhancing the bioavailability of the drug. Diameter and PDI of F2 were 518.6 nm; 0.096 PDI; and a ζ value of -12 mV respectively. The surface visualization of SLN was imaged by microscope in nano-scale, At the Figure 3 was presented the image of particles diameter of 420 nm. According to the un-similar technique in measurement image utilizing this microscope and different manual of SEM and PSA, therefore the precise of using this microscope was more appropriate and also by SEM the environment of SLN such as no-moisture than the DLS method.

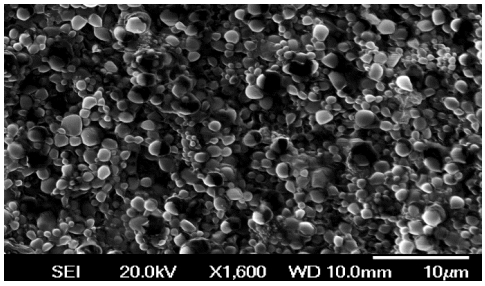

Figure 3. SEM Image of SLN Loading Erythromycin

3.4 Evaluation of pH

Determination of the pH of Solid Lipid Nanoparticles was performed by calculating the pH by utilizing a pH meter. The purpose of using a pH meter is to take an appropriate and accurate value than the other method such as strips-pH. According to the calculating results, in general, it tended that the pH value was slightly lower than neutral (pH of 6.00 – 7.00). The value of pH was presented in Table 3 that the pH values were still appropriate for oral dosage-forms because they are close to neutral (pH 7).

3.5 Physical Stability Test

The stability test of the Solid Lipid Nanoparticle preparation of erythromycin was carried out by the thermal and mechanical

Table 3. Result of Physical Stability and pH Determination

Cycles	Formula 1		Formula 2		Formula 3	
	Visual	pH	Visual	pH	Visual	pH
0	Suspension	5.54±0.024	Suspension	5.62±0.024	Suspension	5.64±0.024
1	Suspension	5.75±0.024	Suspension	5.95±0.024	Suspension	6.07±0.024
2	Suspension	5.80±0.024	Suspension	6.04±0.024	Suspension	6.12±0.024
3	Suspension	5.83±0.024	Suspension	6.17±0.024	Suspension	6.13±0.024
4	Suspension	5.88±0.024	Suspension	6.18±0.024	Suspension	6.20±0.024
5	Aggregation	6.24±0.024	Suspension	6.19±0.024	Aggregation	6.42±0.024
6	Aggregation	6.49±0.024	Aggregation	6.20±0.024	Aggregation	6.88±0.024

methods. The observations were carried out using the Solid Lipid Nanoparticles which were kept for overnight at a chilling and warming temperature side-by-side cycle of 1 week to determine the changing of the physical stability of SLN as organoleptic and value of pH. This process was applied to observe the changes in preparations with different temperatures as an accelerated test. The changes were observed such as organoleptic and pH levels of stability of the SLN loading erythromycin. Based on the observations which were shown in Table 3, organoleptically it was seen that the preparation was quite stable to different storage temperatures which were indicated by the physical appearance in terms of color-changing of SLN and the formation of aggregation. Observations on the pH level revealed a decreasing value in each test cycle. The results of pH measurements for each cycle were statistically analyzed using the IBM SPSS® 25 software. The stability pH data were first tested for requirements, namely the normality test also the Shapiro-Wilk revealed for the sig value > 0.05 which indicates that the data regarding the value of pH were normally distributed to each F. Stability tests were also performed mechanically using a centrifuge. The principle of the process of the centrifugation method is the un-stable SLN will follow the aggregation formed by the time because of the surface interaction of each particle. The suspension/emulsion dosage-form are based on physical stability requirements. This requirement is mimic the real condition of distribution and storage of suspension/emulsion. Centrifugation is rotated int high RPM leads to change in term of the outer surface of the dispersed particles (dis-continues phase) and stimulate coalescence (combined into pellets formation).

3.6 Solubility Test

Solubility testing of Solid Lipid Nanoparticles carrying erythromycin was carried out on the selected formula which purpose to evaluate the enhancing solubility of the SLN loading erythromycin using several solutions for dosage-form manufacturing and the solution which locates in the part of the human body. The results were presented in Table 4. The test solutions used in the solubility test of nanoparticle preparations correspond to some of the solutions in the gastric and intestine. The aims of utilizing the solvent were to see some resistances of the preparation as well as to determine the route for administering drugs that are suitable for the body so that the Solid Lipid Nanoparticle preparation can reach the desired target. The sample solutions used in testing the solubility of Solid Lipid Nanoparticle preparations were distilled water, sodium hydroxide, sodium bicarbonate, acid chloride, artificial gastric solution, and intestine solution. The use of the test solution above aims to condition the same solution as that in the body especially SIF in the intestine as the target location of absorption for erythromycin. As much as 50 mg erythromycin was not soluble in 2 mL of SIF but when in the form of 50 mg of erythromycin loaded by lipid particles was soluble in SIF.

Table 4. Results of Solubility Test of SLN Loading Erythromycin

No.	Solvent	Visualization	Criteria
1	SIF	Clear	Soluble
2	NaOH	Turbid	Not soluble
3	HCl	Clear	Soluble
4	SGF	Clear	Soluble
5	$NaHCO_3$	Turbid	Not soluble
6	Water for injection	Turbid	Not soluble

The increasing in stability and solubility of antibiotics in the form of particles has been widely studied using lipid, PLGA and chitosan-alginate polymers (Bhattacharyya and Reddy, 2019). The small particle size (as the results were presented in the particle size and distribution) impacts to the increasing of surface area as well as solubility (Mardiyanto et al., 2021; Fonseca-Santos et al., 2020). While the stability effect depends on the particle manufacturing process. The encapsulation process is known to provide a coating and protective effect. Encapsulation of erythromycin in this study of lipid particles was around 80% as described and shown in Table 2. This effect can also be exerted by several polymers such as PLGA, chitosan, alginate etc which are semipolar and stearic acid which is non-polar. Since the outer medium is water, nonpolar polymers are last longer and display higher stability than semi-polar ones. The hydrophilic groups of semipolar polymers will interact with water. Instability can be seen from changing in pH due to the exposure of hydroxyl and carboxyl groups in water as a sign

that the particle formation has changed. These data are presented in Tables 2 to 4 and are supported by several Figure 1 to 3.

4. CONCLUSIONS

This research has selected formula of F2 which had a high percentage of encapsulation efficiency of 80.89773±0.11364. The physical characterization of particle formation such as morphology, diameter (particle size) and distribution (PDI) of F2 were spherical 518.6 nm; 0.096 PDI; and a zeta potential value of -12 mV respectively. The particles could increase the stability of erythromycin for up to 5 cycles in terms of the heating-cooling-cycles test and also the solubility in SIF.

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