Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 7, No. 3, July 2022

Research Paper

Formulation and Evaluation of Solid Lipid Nanoparticles Loading Erythromycin Ethylsuccinate by Heating Emulsification and Homogenization Methods

Mardiyanto[1]*, Budi Untari[1], Ady Mara[2], Sadakata Sinulingga[1], Nirwan Syarif[2], Gustina Emilia Ningsih[1]

[1]*Department of Pharmacy, Faculty of Mathematics and Natural Science, Sriwijaya University, Palembang, 30862, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Science, Sriwijaya University, Palembang, 30862, Indonesia*
***Corresponding author**: mardiyanto@mipa.unsri.ac.id*

Abstract
The pandemic period has caused antibiotics highly recommended to cure infections. The use of macrolide antibiotics has greatly increased recently due to outbreaks of diseases that attack the human respiratory tract all of part of the world. One member of the macrolide group is erythromycin ethyl succinate which has low solubility in water. Therefore, this study aims to convert erythromycin ethyl succinate into lipid nanoparticles in an attempt to increase solubility. The method for the formation of nanoparticles is heating emulsification and homogenization. The results obtained in the form of formula 1 (F1) showed the percent encapsulation of 85.688±0.30641. The physical properties were that it has a size of 398.9±1.4 nm, a PDI of 0.3895±0.0015, and zeta potential of -17.45±0.15 mV respectively. The stability was determined by an accelerated test by the influence of extreme temperature and mechanics affecting the stability of the particles as an indication of decreasing the pH and particle precipitation. The solubility of erythromycin ethyl succinate in the form of lipid nanoparticles was increased in a comparison with the pure substance of erythromycin ethyl succinate.

Keywords
Formulation, Lipid-Nanoparticles, Stearic-Acid, Erythromycin, Solubility

Received: 18 March 2022, Accepted: 23 June 2022
https://doi.org/10.26554/sti.2022.7.3.320-326

1. INTRODUCTION

The use of modifying the structure of drug substances to increase solubility is a study that researchers in the pharmaceutical field are currently actively undertaking. The drug ingredients are in the form of synthetic and semi-synthetic materials from antibiotics, antidiabetics, and anti-inflammatories (Fini et al., 2005; Pignatello et al., 2017) including the potent natural ingredients (Sandi et al., 2018; Untari et al., 2019; Watkins et al., 2015). More than 70% of the new medicinal ingredients have a problem with solubility. Solubility is related to bioavailability which can provide an overview of the concentration of drug compounds entering the blood. Reducing the physical size of drug compounds by forming nanoparticles also can increase the solubility. Research on forming nanometer-sized drug compounds is still not intensively carried out thus the research gap is the preparation of the nanoparticles of drug compounds to increase the solubility of drug compounds as one of the requirements for the effectiveness of therapy in the human body.

Previous studies reported that the formation of lipid particles was determined by the use of surfactants, cosurfactants, and lipids using the emulsification process. The formation of these lipid particles has succeeded in carrying glibenclamide, rifampicin, erythromycin, ibuprofen, and others (Mahmood et al., 2020; Qiao et al., 2022; Rupenagunta et al., 2011). The amount of lipid used is adjusted to the interaction capability of the surfactant. Many types of lipids have been tested, such as stearic acid, but not many studies have considered the molecular size of the drug substance that interacts with lipids and the physical properties of stearic acid as a lipid that at ordinary temperatures is a solid (Gonçalves et al., 2016; Sarathchandiran, 2012).

Lipid particles are also classified as polymeric particles. The preparation of nanometer-sized lipid particles is not easy compared to other polymeric particles but it proved to be more stable and resistant in water. The preparation of these lipid particles requires an emulsification process and the Tween and Span as an emulsifier is often used together with polyethylene glycol (PEG) as a co-emulsifier so that the resulting product is more stable. The lipids used range from liquid ones such as virgin coconut oil (VCO) or solid ones such as stearic acid and stearyl alcohol (Mardiyanto et al., 2021). Most lipids are

solid at room temperature, so hot emulsification processes have been considered (Fonseca-Santos et al., 2020; Rupenagunta et al., 2011).

Due to stability in water, lipid nanoparticles have been considered carriers of antibiotics because antibiotics are generally not as stable in water as macrolide antibiotics. Erythromycin is an example of a macrolide class of antibiotics. Over time, erythromycin has been synthesized in combination with ethyl succinate. The molecular structure of erythromycin ethyl succinate is shown in Figure 1. The solubility of erythromycin ethyl succinate in water does not increase and neither does stability. Erythromycin ethyl succinate oral dosage form is still free from water in the form of dry powder (Cao et al., 2019; Qiao et al., 2022). Therefore, the focus of this research is to increase the solubility of erythromycin ethyl succinate in water and also its stability in the water as a novelty that will be useful for the development of drug dosage forms.

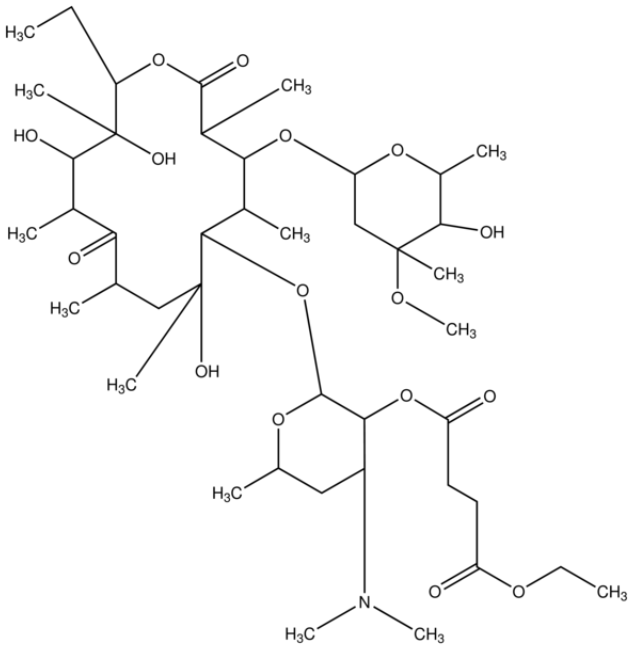

Figure 1. Molecule Composition of Erythromycin-Ethylsuccinate

2. EXPERIMENTAL SECTION

2.1 Materials

Erythromycin ethyl succinate and polyvinyl alcohol (PVA) were purchased from Sigma-Aldrich®. Stearic acid, polysorbate-80 (Tween-80), polyethylene glycol 400 (PEG-400), ethanol, ethyl acetate, and hydrochloric acid were purchased from Merc-KGaA®. Water for injection was purchased from Otsuka®.

2.2 Methods

2.2.1 Lipid Nanoparticle Formulation

The planned formula is for 100 mL of the final volume of the product. The amount of erythromycin ethyl succinate used follows a single-use dose of 250 mg (Basu and Smith, 2021; Martingano et al., 2020). The amounts of PVA, polysorbate 80, and PEG-400 are based on previous studies where surfactant and cosurfactant form emulsion. The amount of stearic acid was also based on a previous study with the best formula using 1 g. The planned variation point was the amount of stearic acid from 1 to 2 g (Bhattacharyya and Reddy, 2019; Mardiyanto et al., 2021; Rupenagunta et al., 2011; Sarathchandiran, 2012). The composition of the ingredients for the formulation of lipid nanoparticles can be seen in Table 1.

Table 1. Composition of Materials for Formulation of Lipid Nanoparticles

Ingredient	Function	Amount (g)		
		F1	F2	F3
Erythromycin	Active compound	0.25	0.25	0.25
Stearic acid	Lipid	1	1.5	2
Tween 80	Surfactant	1	1	1
PEG 1000	Cosurfactant	0.5	0.5	0.5
PVA	Stabilizer	1	1	1
WFI	Media	Add 100 mL	Add 100 mL	Add 100 mL

2.2.2 Dissolving of Lipid and Water Phase

As amounts of 1, 1.5, and 2 g of stearic acid were weighed carefully and placed in a Beaker glass. Because the melting process of stearic acid requires heat, in this study, the stearic acid liquefaction was carried out at a temperature of 75°C using a water bath (Memmert®). Furthermore, the water phase was also prepared including polysorbate 80 and PEG-400 were weighed as much as 1 g and 0.5 g in a Beaker glass, and also as amounts of 2.5 g of PVA were weighed carefully and put in an Erlenmeyer which already contained 100 mL of water for injection and then stirred at 60°C for 200 RPM overnight using hot plate stirrer (IKA® C-MAG). The 2.5% PVA was obtained after filtration using a 0.78 μm filter. Furthermore, all the materials that have been prepared were placed in a water bath which was set at 60°C (Mardiyanto et al., 2021).

2.2.3 Preparation of Erythromycin Ethylsuccinate Loaded Lipid Nanoparticles

The water bath was occupied by the glass Beaker and Erlenmeyer contains the aqueous phase and the lipid phase of the lipid nanoparticles except for erythromycin ethyl succinate and then the temperature was increased to 75°C for 3 hours (Memmert®). The ultrasonicator probe (Shalom Ultrasonics®) was immersed in water for injection at the same temperature and treatment time. The temperature was controlled with a thermometer. In a hot water bath, polysorbate 80 and PEG-400 were combined and stirred until homogeneous. To the

stearic acid which has completely melted, erythromycin ethyl succinate was added and stirred until homogeneous for no more than 10 minutes. Furthermore, at a controlled temperature, the aqueous phase, and the lipid phase were mixed and immediately transferred to the ultrasonicator probe which has been conditioned at 75°C to be homogenized for 2 minutes. Then it was left on a stirring plate at 40°C at 1,000 RPM for 3 hours (Fonseca-Santos et al., 2020; Ikeuchi-Takahashi et al., 2016).

2.2.4 Selection of The Best Formula Based on Loading Capacity

The loading capacity was determined by calculating the encapsulation efficiency (%EE). The amount of unencapsulated erythromycin was determined using spectrophotometry. The amount of unencapsulated erythromycin will use to determine the percentage of encapsulation. The unencapsulated amount of erythromycin was as a liquid supernatant when the lipid nanoparticle dispersion was centrifuged at 13,000 RPM for 15 min. Absorbance measurements were carried out at a wavelength of 212 nm using a UV-Vis spectrophotometer (UV-1700 Shimadzu®) (Mardiyanto et al., 2021).

2.2.5 Determination of The Acidity of Lipid Nanoparticles

Determination of the acidity of the lipid nanoparticles product from the three prepared formulas was carried out using a pH meter (Luthron® pH Electrode). The dispersions of the three formulas were placed at room temperature. The probe of the pH meter was calibrated and placed in water for injection. The pH meter probe was immersed in the sample alternating with water for injection (Ikeuchi-Takahashi et al., 2016). A dry probe could be ensured that residual water was not affecting the pH measured. Each sample was measured in triples and the results were tabled.

2.2.6 Determination of Size, PDI, Zeta-Potential, and Morphology Particles

Determination by dynamic scattering of light was for hydrodynamic properties that could determine size, uniformity, and zeta potential. The particle size analyzer (PSA) by Horiba® SZ100 was a tool that was used with the dynamic principle of scattering light. These three parameters could be measured with a PSA detector with two types of narrow and wide angles. First, the detector position needs to be considered and the refractive index of light when passing through the water as the medium can be selected from PSA. Samples in a dilute state were also a factor to set. The 100 μL sample was put in a 1.5 mL PSA cuvette and the sample was diluted 10 times with water for injection. In addition, the measurement without water is to determine the morphology of the lipid nanoparticles. The microscopic method is the method used to determine morphology. Scanning electron microscopy (SEM) by EVO-HD®1600 was used for the 200 μL sample in 1 mL water on top of the SEM holder dried under ambient conditions. Very thin layer coated with gold for 15 minutes with scattering voltage 19 V and contact resistance 1 mΩ to result from 0.2 μm to 5 μm thickness of gold. For capturing of image, the setting of ejection of electrons was 20 kV and magnification of 10.000 times (Mardiyanto et al., 2021).

2.2.7 Determination of Stability and Solubility of Product

The method used to determine stability is the accelerated method. The first was given to a sample of a combination of hot and cold temperatures every 24 hours. One cycle was a combination of hot and cold. The test was continued for up to 6 cycles. Observations were the physical changes of the sample organoleptically for each cycle as well as changes in pH. Furthermore, it was compared to a test that uses mechanics in the form of centrifugation. Samples were centrifuged at a medium speed of 3,000 RPM but with a long time of 3 hours. The observation was whether or not a precipitate formed. The solubility evaluation of erythromycin ethyl succinate which has been in the form of nanoparticles was compared to the solubility of pure erythromycin which can indicate a change in solubility. The observation was to record the optical density at 520 nm. Turbidity was detected as optical density. For the solubility test, three products from the three formulas were pipetted at 400 μL each and then diluted to 1 mL of water for injection, 1 M HCl, 1 M NaOH, sodium bicarbonate, SIF, and SGF (Mardiyanto et al., 2021).

3. RESULTS AND DISCUSSION

3.1 Lipid Nanoparticle Formulation for Loading Erythromycin

The medicinal ingredient selected in this study was a medicinal ingredient whose use was increasing in the world along with the respiratory tract epidemic (Blumenberg et al., 2020). The aim was to improve encapsulation then erythromycin ethyl succinate was the right choice according to the problem of solubility. The formulation aspect is involved in the solubility. Solubility is related to the dissolution and absorption of an active compound (Jain and Patil, 2015; Kalepu and Nekkanti, 2015; Pignatello et al., 2017; Rapalli et al., 2021). So that in this study the effect of variations in stearic acid that will carry erythromycin will be known. Three variations of stearic acid were based on previous studies and were attempted to be increased from 1 to 1.5 and 2 g for 100 mL product (Sarathchandiran, 2012). While the amount of surfactant and cosurfactant was based on the usual use to prepare a pharmaceutical emulsion preparation. In previous research, the preparation of the water and oil phases were separated from each other and the temperature was not controlled, especially in the water phase, because actually, stearic acid needed heat to melt. There were also nanoparticle lipids that were not made with stearic acid such as liquid coconut oil so the temperature was not used in the preparation process. When polysorbate 80, PEG-400, and PVA were mixed, a clear and viscous solution was formed. Furthermore, when erythromycin ethyl succinate was mixed into melted stearic acid, a turbid mixture occurs and after 1 minute the mixture becomes clear. If turbidity persists, it indicates that there has been no interaction between erythromycin ethyl succinate and

stearic acid. Especially for antibiotics as is the case with erythromycin ethyl succinate, heating was not involved during this preparation process. Therefore, erythromycin ethyl succinate was added immediately to the formation of lipid nanoparticles. The concentration of surfactant 0.3 to 1.5% (w/w) and cosurfactant 0.1 to 0.8% (w/w) are the common concentration for emulsification. Previous research indicated that surfactant 1% and cosurfactant 0.5% produced a stable emulsion.

3.2 Preparation of Lipid Nanoparticles Loading Erythromycin Etilsuccinate

Increasing the temperature from 60 to 75°C is to decrease the viscosity of the mixture. The decrease in viscosity makes it easier for the material to interact. In this mixing process, the drug substance is placed in the core position. So that the medicinal ingredients must be interacted with stearic acid and then be coated with surfactants and cosurfactants (Fonseca-Santos et al., 2020). The formation of interactions takes time, so a stirring process is needed. Particle size reduction and homogenization are also required by using a tool whose probe has also been soaked in hot water otherwise the stearic acid could simultaneously freeze in the cold metal probe and the stearic acid could escape from the mixture. Results of the formation of lipid nanoparticles was displayed in Table 2. In the other research, besides ultrasonicator, ultra turrax was also used. Ultraturax also has a metal probe where stearic acid could also be influenced. Related to the structure of erythromycin ethyl succinate, especially the hydrophobic part, they can interact with the hydrophobic part of drugs. The hydrophobic part of surfactants also interacts with stearic acid. The surfactant can be coated by the cosurfactant and the gap in the stearic acid that is not filled by the surfactant is also covered by the cosurfactant. The hydrophilic part of the surfactant and cosurfactant can interact with water as a medium. This creates a stable emulsion. Figure 2 showed the image of the product which was prepared.

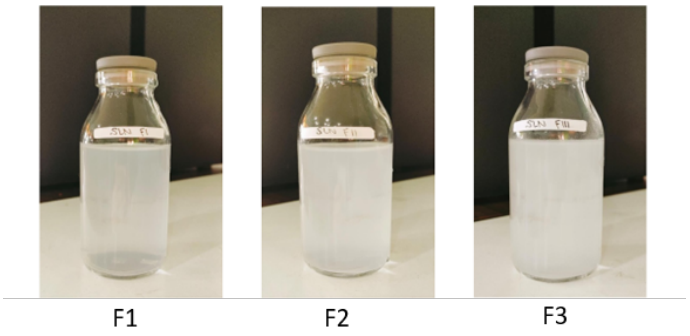

Figure 2. Lipid Nanoparticles of F1, F2, and F3

3.3 Determination of Percent Efficiency of Encapsulation

Determination of the percentage of encapsulation is to determine its efficiency with the term indirect determination. There is a more valid method, namely direct determination. But expecting all the nanoparticles to be lysed and the drug released is difficult, so the accuracy of the data is questionable. That's why many experts do it indirectly. Centrifugation conditions have been optimized as in previous studies. The turbidity of the supernatant needs to be checked before the spectrophotometry process is carried out (Surendra et al., 2020).

Table 2. Results of Product Preparation of Lipid Nanoparticles Loading Erythromycin Ethylsuccinate

Results		
F1	F2	F3
Transparent, light emulsion,and flavorless	Turbid, light emulsion, and flavorless	Turbid, emulsion, and flavorless

Encapsulation of a product that is closer to the amount of drug added to the formula is estimated to be the better the interaction ability of the polymer with the drug substance. At least show a value above 50%. The percent value of encapsulation efficiency can be determined using a UV-Vis spectrophotometer instrument. UV-Vis spectrophotometry is a method used to measure absorption resulting from chemical interactions between electromagnetic radiation and molecules or atoms of a chemical substance in the ultraviolet and visible regions of light. The results obtained were more than 50% and close to 86% could be seen in Table 3. The amount of stearic acid of more than 1 g for each formula was an indication of inefficiency because it looks like the emulsion was more turbid and did not have an impact on increasing %EE. An excess amount of stearic acid causes problems with the physical particles. An excess amount of stearic acid can combine each other and attract other stearic acids that have interacted in the formation of particles. As a result, the medicinal ingredients will come out and not be completely encapsulated. This released drug substance will be detected if the method of determining the encapsulation is indirect (Gupta et al., 2016; Oliveira et al., 2016; Pignatello et al., 2018) as an illustration in Figure 3.

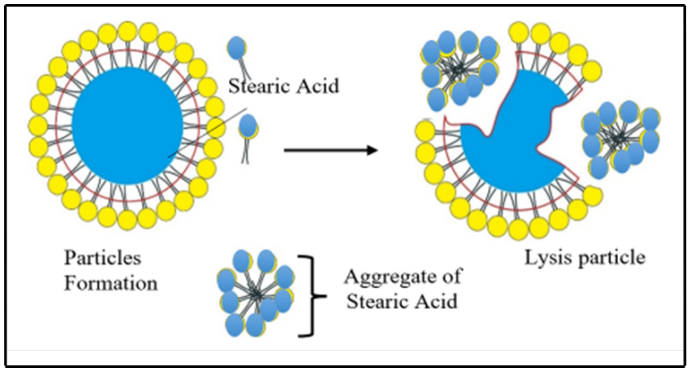

Figure 3. The Effect of Stearic Acid Aggregates on The Encapsulating Particles of The Drug Substance

Table 3. Results of Determination Indirect Efficiency (%EE)

Formula	Stearic Acid (g)	Mean of %EE±SD (%)	CV (%)
F1	1	85.688±0.30641	0.35759
F2	1.5	81.205±0.24494	0.30164
F3	2	71.705±0.08165	0.11386

3.4 Results of Determination of PSA and Morphology

The higher %EE value is taken as the preferred formula, namely F1. Because in the pharmaceutical field, the first thing to consider is the number of drugs, especially antibiotics that are associated with cases of bacterial resistance if the amount in the preparation does not match the therapeutic dose. Furthermore, this product was characterized physically in the form of size, PDI, and zeta potential using the PSA tool (Mardiyanto et al., 2021). The results of the particle diameter measurement in the selected formula were obtained at 398.9±1.4 nm. The results of this measurement indicate that the particle diameter size obtained has entered the particle size range. This is following the average range of SLN lipid particle size is 20–1000 nanometers. The large size is probably due to the small zeta potential value, resulting in agglomeration between particles. This can also be caused by the occurrence of physical instability in erythromycin particle preparations and causes an increase in particle size during sample storage. In addition, a vortex system should be carried out to prevent the formation of aggregates between particles before carrying out the test.

The particle size distribution parameter is also determined by the value of the polydispersity index (PDI) which is used to determine the level of size uniformity. The PDI value can affect the stability of the particle erythromycin preparation. The results of the PDI value of the best formula for the particles erythromycin preparation obtained a PDI value of less than 0.5, respectively 0.3895±0.0015 which indicated that there were 62% uniform particles and belong to the monodisperse dispersion type. Results of PSA were presented in Table 4.

Table 4. Results of Measurement Using PSA

Properties	Mean±SD	%CV
Size	398.9±1.4 nm	0.35096
PDI	0.3895±0.0015	0.38511
Zeta potential	-17.45±0.15	0.85961

The results of observations with an electron microscope, namely SEM, show a smaller size than PSA. PSA is carried out in an aqueous environment and the observed trend is the hydrodynamic size. Unlike the microscope, the technique used is without water. The SEM results in Figure 4 indicate that the particles are spherical and have a size of about 300 nm using the Surface-Topology® program.

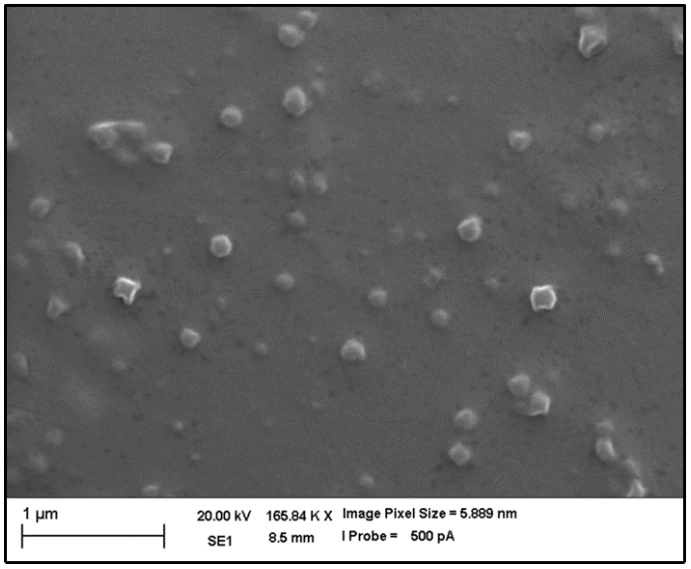

Figure 4. Morphology Image of Erythromycin Particles

3.5 Result of Determination of pH, Stability, and Solubility

Lipid as the structure does not interact with water but lipid is influenced by temperature (Almanassra et al., 2021; Chantaburanan et al., 2017). Actually, the pH of the medium is not estimated to have much effect on lipids. But because now on the surface of the particle there is also a stabilizer and cosurfactant, so the pH needs to be determined as an indication that some of the material is released from the particle and causes a decrease in pH. The pH obtained from the preparation as a whole was close to neutral and none was above 7 as shown in Table 5.

Table 5. Results of Determination of pH Particles

Formula	Stearic Acid (g)	Mean pH±SD (%)	CV (%)
F1	1	6.45±0.0047	0.0730486
F2	1.5	6.27±0.0047	0.0752237
F3	2	6.18±0.0047	0.0730486

In general, F1, F2, and F3 were stable in water for 30 days at room temperature. To see if this product is stored in uncontrolled conditions in the future, an accelerated stability test was carried out. This means that the product was given extreme conditions. There were two conditions studied, namely extreme temperatures and mechanics. The temperature was made hot and cold alternately every 24 hours. One cycle was hot and cold. So, for one cycle it has taken 2 days. The study was conducted for 6 cycles so it takes 12 days. With extreme conditions, the results showed that on day 10^{th} or cycle 5^{th} there was physical instability and changes in pH as an indication that surfactants and cosurfactants, and stabilizers were no longer able to protect particles. The results can be seen

Table 6. Results of Changes in Physic and Acidity by Thermal

Cycle	Formula 1 Organoleptic	pH	Formula 2 Organoleptic	pH	Formula 3 Organoleptic	pH
0	Clear light emulsion, no sedimentation	6.45	Clear light emulsion, no sedimentation	6.27	Clear emulsion, no sedimentation	6.18
1	Clear light emulsion, no sedimentation	6.44	Clear light emulsion, no sedimentation	6.24	Clear emulsion, no sedimentation	6.18
2	Clear light emulsion, no sedimentation	6.42	Clear light emulsion, no sedimentation	6.23	Clear emulsion, no sedimentation	6.15
3	Clear light emulsion, no sedimentation	6.42	Clear light emulsion, no sedimentation	6.18	Clear emulsion, no sedimentation	6.14
4	Clear light emulsion, no sedimentation	6.40	Clear light emulsion, no sedimentation	6.16	Clear emulsion, no sedimentation	6.09
5	Clear light emulsion, less sedimentation	6.41	Turbid light emulsion, sedimentation	6.10	Turbid light emulsion, sedimentation	6.01
6	Clear light emulsion, sedimentation	6.43	Turbid light emulsion, sedimentation	6.02	Turbid light emulsion, sedimentation	5.92

in Table 6. It is the same with mechanical administration in the form of centrifugation for 3 hours. The preparations are generally stable when centrifuged at 3,000 RPM for less than one hour. However, in extreme conditions in the form of a long time, which is more than 3 hours, precipitation of particles occurred in F1, F2, and F3. All samples are insoluble in water and solutions with a pH above 7 such as sodium bicarbonate and sodium hydroxide. The sample appears to give a clear solution in an acidic solution. In the simulated gastrointestinal fluid (SGF) solution, a comparison was made with pure erythromycin ethyl succinate that in an amount comparable to the formula of erythromycin ethyl succinate nanoparticles was insoluble in SGF. This means in this study it was successful in making erythromycin ethyl succinate soluble when it was in the form of lipid nanoparticles.

4. CONCLUSIONS

Research that intends to bring erythromycin ethyl succinate into lipid nanoparticles has been successfully carried out with %EE of 85.6%. The physical characterization of nanoparticles indicates that they can be used as drug preparations. The particle size was 398.9 nm, PDI was 0.3895, and zeta potential was -17.45 mV respectively. The solubility of erythromycin ethyl succinate in the form of lipid nanoparticles was increased in a comparison with the pure substance of erythromycin ethyl succinate.

5. ACKNOWLEDGMENT

Authors conducted this research by utilizing the research funding of "Penelitian Unggulan Kompetitif Sriwijaya University" for the academic year of 2022. The formation of particles also their evaluation were conducted at the Laboratory of Technology-Pharmacy at the Faculty of Science UNSRI, Centre of Nanofabrication of UII, and also the Laboratory of Particles Chemistry of UGM Yogyakarta.

REFERENCES

Almanassra, I. W., E. C. Okonkwo, O. Alhassan, M. A. Atieh, V. Kochkodan, and T. Al-Ansari (2021). Stability and Thermophysical Properties Test of Carbide-Derived Carbon Thermal Fluid; a Comparison Between Functionalized and Emulsified Suspensions. *Powder Technology*, **377**; 415–428

Basu, S. and S. Smith (2021). Macrolides for The Prevention and Treatment of Feeding Intolerance in Preterm Low Birth Weight Infants: a Systematic Review and Meta-Analysis. *European Journal of Pediatrics*, **180**(2); 353–378

Bhattacharyya, S. and P. Reddy (2019). Effect of Surfactant on Azithromycin Dihydrate Loaded Stearic Acid Solid Lipid Nanoparticles. *Turkish Journal of Pharmaceutical Sciences*, **16**(4); 425

Blumenberg, V., M. L. Schubert, E. Zamir, S. Schmidt, R. Rohrbach, P. Waldhoff, D. Bozic, H. Pock, E. Elinav, and C. Schmidt (2020). Antibiotic Therapy and Low Gut Microbiome Diversity is Associated with Decreased Response and High Toxicity in BCP-ALL and DLBCL Patients after Treatment with CD19. CAR T-Cells. *Blood*, **136**; 33–34

Cao, Y., S. Xuan, Y. Wu, and X. Yao (2019). Effects of Long-Term Macrolide Therapy at Low Doses in Stable COPD. *International Journal of Chronic Obstructive Pulmonary Disease*, **14**; 1289

Chantaburanan, T., V. Teeranachaideekul, D. Chantasart, A. Jintapattanakit, and V. B. Junyaprasert (2017). Effect of Binary Solid Lipid Matrix of Wax and Triglyceride on Lipid Crystallinity, Drug-Lipid Interaction and Drug Release of Ibuprofen-Loaded Solid Lipid Nanoparticles (SLN) for Dermal Delivery. *Journal of Colloid and Interface Science*, **504**; 247–256

Fini, A., J. R. Moyano, J. M. Ginés, J. I. Perez-Martinez, and

A. M. Rabasco (2005). Diclofenac Salts, II. Solid Dispersions in PEG6000 and Gelucire 50/13. *European Journal of Pharmaceutics and Biopharmaceutics*, **60**(1); 99–111

Fonseca-Santos, B., P. B. Silva, R. B. Rigon, M. R. Sato, and M. Chorilli (2020). Formulating SLN and NLC as Innovative Drug Delivery Systems for Non-Invasive Routes of Drug Administration. *Current Medicinal Chemistry*, **27**(22); 3623–3656

Gonçalves, L., F. Maestrelli, L. D. C. Mannelli, C. Ghelardini, A. Almeida, and P. Mura (2016). Development of Solid Lipid Nanoparticles as Carriers for Improving Oral Bioavailability of Glibenclamide. *European Journal of Pharmaceutics and Biopharmaceutics*, **102**; 41–50

Gupta, B., B. K. Poudel, S. Pathak, J. W. Tak, H. H. Lee, J. H. Jeong, H. G. Choi, C. S. Yong, and J. O. Kim (2016). Effects of Formulation Variables on The Particle Size and Drug Encapsulation of Imatinib-Loaded Solid Lipid Nanoparticles. *Aaps Pharmscitech*, **17**(3); 652–662

Ikeuchi-Takahashi, Y., C. Ishihara, and H. Onishi (2016). Formulation and Evaluation of Morin-Loaded Solid Lipid Nanoparticles. *Biological and Pharmaceutical Bulletin*, **39**(9); b16–00300

Jain, G. and U. K. Patil (2015). Strategies for Enhancement of Bioavailability of Medicinal Agents with Natural Products. *International Journal of Pharmaceutical Sciences and Research*, **6**(12); 5315–5324

Kalepu, S. and V. Nekkanti (2015). Insoluble Drug Delivery Strategies: Review of Recent Advances and Business Prospects. *Acta Pharmaceutica Sinica B*, **5**(5); 442–453

Mahmood, H. S., M. Alaayedi, M. M. Ghareeb, and M. M. M. Ali (2020). The Enhancement Solubility of Oral Flurbiprofen by Using Nanoemelsion as Drug Delivery System. *International Journal of Pharmaceutical Research*, **12**; 1612–1619

Mardiyanto, M., N. A. Fithri, A. Amriani, H. Herlina, and D. P. Sari (2021). Formulation and Characterization of Glibenclamide Solid Lipid Submicroparticles Formated by Virgin Coconut Oil and Solid Matrix Surfactant. *Science and Technology Indonesia*, **6**(2); 58–66

Martingano, D., S. Singh, and A. Mitrofanova (2020). Azithromycin in The Treatment of Preterm Prelabor Rupture of Membranes Demonstrates a Lower Risk of Chorioamnionitis and Postpartum Endometritis with an Equivalent Latency Period Compared with Erythromycin Antibiotic Regimens. *Infectious Diseases in Obstetrics and Gynecology*, **2020**

Oliveira, M. S., S. V. Mussi, D. A. Gomes, M. I. Yoshida, F. Frezard, V. M. Carregal, and L. A. Ferreira (2016). α-Tocopherol Succinate Improves Encapsulation and Anticancer Activity of Doxorubicin Loaded in Solid Lipid Nanoparticles. *Colloids and Surfaces B: Biointerfaces*, **140**; 246–253

Pignatello, R., V. Fuochi, G. Petronio Petronio, A. S. Greco, and P. M. Furneri (2017). Formulation and Characterization of Erythromycin–Loaded Solid Lipid Nanoparticles. *Biointerface Research in Applied Chemistry*, **7**(5); 32–39

Pignatello, R., A. Leonardi, V. Fuochi, G. Petronio Petronio, A. S. Greco, and P. M. Furneri (2018). A Method for Efficient Loading of Ciprofloxacin Hydrochloride in Cationic Solid Lipid Nanoparticles: Formulation and Microbiological Evaluation. *Nanomaterials*, **8**(5); 304

Qiao, D., W. Shi, M. Luo, F. Jiang, and B. Zhang (2022). Polyvinyl Alcohol Inclusion Can Optimize The Sol-Gel, Mechanical and Hydrophobic Features of Agar/Konjac Glucomannan System. *Carbohydrate Polymers*, **277**; 118879

Rapalli, V. K., S. Sharma, A. Roy, A. Alexander, and G. Singhvi (2021). Solid Lipid Nanocarriers Embedded Hydrogel for Topical Delivery of Apremilast: In-Vitro, Ex-Vivo, Dermatopharmacokinetic and Anti-Psoriatic Evaluation. *Journal of Drug Delivery Science and Technology*, **63**; 102442

Rupenagunta, A., I. Somasundaram, V. Ravichandiram, J. Kausalya, and B. Senthilnathan (2011). Solid Lipid Nanoparticles-A Versatile Carrier System. *Journal of Pharmaceutical Sciences*, **4**(7); 2069–2075

Sandi, S., F. Yosi, and M. L. Sari (2018). Preparation and Characterization of Bio-\ Polymeric Nano Feed Incorporating Silage-Derived Organic-Acids and The Polar Fraction of Papaya Leaf Extract. *Journal of Physics: Conference Series*, **1095**; 012024

Sarathchandiran, I. (2012). A Review on Nanotechnology in Solid Lipid Nanoparticles. *Journal of Pharmaceutical Development Technology*, **2**(1); 45–61

Surendra, B., M. N. Kumar, and P. Iriventi (2020). Formulation and Evaluation of Caffeine-Loaded Solid Lipid Nanoparticles to Treat Clinical Mastitis. *Asian Journal of Pharmaceutical and Clinical Research*, **23**(3); 79–85

Untari, B., D. P. Wijaya, M. Mardiyanto, H. Herlina, and A. Firana (2019). Physical Interaction of Chitosan-Alginate Entrapping Extract of Papaya Leaf and Formation of Submicron Particles Dosage form: New Dossage form to Inhibit The Dengue Diseases. *Science and Technology Indonesia*, **4**(3); 64–69

Watkins, R., L. Wu, C. Zhang, R. M. Davis, and B. Xu (2015). Natural Product-Based Nanomedicine: Recent Advances and Issues. *International Journal of Nanomedicine*, **10**; 6055

