In-Vitro Dissolution and Characterization of Self-Emulsifying Drug Delivery System of Artemisinin for Oral Delivery

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Abstract. Artemisinin is a compound extracted from Artemisia Annua. Artemisinin is used globally as the first-line antimalarial drug. Despite its high efficacy against the malaria parasite, artemisinin has low bioavailability because it has low solubility in water. This present study was conducted to prepare and characterize the self-emulsifying drug delivery system of artemisinin to increase the dissolution profile of artemisinin. The stability of the resulting emulsion was observed visually for 6 hours. Droplet size, polydispersity index, and zeta potential of the emulsion were measured using Nano Particle Analyzer. The optimum formulation was evaluated with the dissolution test and compared with the artemisinin crystal. Several formulations have good stability of the resulting emulsions where no creaming or flocculation was formed during the observation. Droplet sizes of the resulting emulsions ranged from 114.17-247.93 nm and the polydispersity index of the emulsions ranged from 0.35- 0.56. Zeta potential values of the selected formulations were found in the range of -23.23 to -2.33 mV. Fourier Transform Infrared Spectroscopy spectra of self-emulsifying drug delivery system showed the presence of artemisinin in the formulation with lactone and peroxide peaks. The dissolution of artemisinin in the self-emulsifying drug delivery system was significantly increased compared to artemisinin crystal. Artemisinin was released up to 98.6 % in 150 minutes in self-emulsifying drug delivery system formulation.

1. Introduction
Malaria is one of humanity's oldest diseases and potentially life-threatening disease which is caused by infection with Plasmodium parasites. Plasmodium falciparum is the most dangerous among other Plasmodium parasite species. It has developed resistance to almost all antimalarial drugs such as quinine, chloroquine, proguanil, sulfadoxine-pyrimethamine, mefloquine, atovaquone, and amodiaquine [1]. The increasing resistance of malaria parasites to drugs increases the burden of the disease and the need to find and develop a new effective antimalarial drug.

Artemisinin is the most effective antimalarial medicine these days. Artemisinin is a sesquiterpene lactone antimalarial with a peroxide linkage [2]. Artemisinin-based combination therapies (ACT) are currently recommended as treatments for malaria globally by the World Health Organization. Over the period 2010-2017, countries in the world have procured 2.74 billion treatment courses of artemisinin-based combination therapy [3]. Artemisinin compound can reduce Plasmodium parasite load during the first 3 days of treatment, and the other antimalarial drug as a partner drug can eliminate the...
remaining Plasmodium parasites [4]. Furthermore, the activity of artemisinin against Leishmania, Schistosoma, Toxoplasma, and against a variety of unrelated tumor cell lines, such as colon, breast, lung cancers, leukemias, and pancreatic cancer have also been reported [5-8]. Despite its activity against parasites, artemisinin has poor bioavailability limiting its efficacy. Artemisinin has low solubility in water and high first-pass metabolism. It can lead to poor absorption through intravenous injection or oral administration [9].

Many strategies have been applied to enhance artemisinin solubility, dissolution properties, and efficacy. Artemisinin-loaded conventional liposomes, artemisinin–curcumin-loaded conventional liposomes, artemisinin-loaded PEGylated liposomes, artemisinin–curcumin-loaded PEGylated liposomes appeared to have an immediate antimalarial effect [10]. The lipid-based drug delivery system of antimalarial artemether and lumefrantirine has been reported to complete and faster in vitro drug release compared to marketed tablets [9]. Lipid-based drug delivery system for oral delivery of β arteether has been reported had an antimalarial efficacy comparable to the intramuscular oily solution of arteether and significantly higher than oily solution of β arteether. Lipid-based drug delivery system is a promising method for improving the oral delivery issue of antimalarial drugs [11].

Self Emulsifying Drug Delivery System (SEDDS) is one of the lipid-based drug delivery systems to improve the dissolution profile and bioavailability of the drug that is poorly soluble in water. SEDDS is an isotropic system and thermodynamically stable solution containing drug, oil, surfactant, and co-surfactant mixtures that will spontaneously form oil-in-water (O/W) emulsions when mixed with water or gastrointestinal fluid under gentle stirring [12]. It has been reported SEDDS can significantly improve the release profile of poorly soluble drugs such as aceclofenac, indapamide, atorvastatin, ezetimibe, losartan potassium [13-16]. SEDDS also well known can avoid the hepatic first-pass metabolism effect by using an intestinal lymphatic pathway and lead to an increase in the bioavailability of the drug [16].

In this study, attempts were made to prepare and characterize the SEDDS of artemisinin for oral administration. SEDDS is a potential method for improving the dissolution profile and bioavailability of artemisinin which has poor solubility in water and high pass first metabolism.

2. Materials and Methods

2.1. Materials

The materials used in this research were demineralized water, artemisinin obtained from Organic Herb Inc., China with a purity of 99.11%, oleic acid (PT. Cisadane Raya Chemicals, Indonesia), ethanol pro analysis (E. Merck, Germany), 96% ethanol (PT. Brataco, Indonesia) , tween 80 (E. Merck, Germany), polyethylene glycol 400 (PEG 400) (PT. Brataco, Indonesia), sodium hydroxide (E. Merck, Germany), size 0 hard gelatin capsules (Capsugel, United State).

2.2. Preparation of Artemisinin Self Emulsifying Drug Delivery System

Artemisinin (2% w / w) was dissolved in a mixture of oil, surfactant, and co-surfactant with concentrations of oil, surfactant, and co-surfactant as can be seen in Table 1. The mixture was stored at room temperature (25-26°C) until the time of testing.

| Samples | Surfactant Concentration (%) | Co-surfactant Concentration (%) | Oleic Acid Concentration (%) |
|---------|-----------------------------|--------------------------------|-----------------------------|
| F1      | 50                          | 5                              | 45                          |
| F2      | 50                          | 10                             | 40                          |
| F3      | 50                          | 15                             | 35                          |
| F4      | 50                          | 20                             | 30                          |
| F5      | 50                          | 25                             | 25                          |
| F6      | 60                          | 5                              | 35                          |
2.3. Physical stability observation
Physical stability of all formulations was performed by visual observation. 0.01 mL of each formulation was added into 10 mL pH 6.8 phosphate buffer in the reaction tube then gently shaken at 37°C. All formulations were observed every 15 minutes for 6 hours. The most stable formulations were then selected for particle size, polydispersity index, and zeta potential determination.

2.4. Particle size measurement
The particle size and polydispersity index were measured by the Dynamic Light Scattering method using the Nanoparticle Analyzer Horiba SZ-100. Samples were measured after dispersion into 10 mL pH 6.8 phosphate buffer at a sample: medium ratio of 1: 1000 v/v. The test was carried out at 37°C. Measurements were performed in triplicate.

2.5. Zeta potential measurement
Zeta potential was measured using the Nanoparticle Analyzer Horiba. The samples were measured after dispersion into pH 6.8 phosphate buffer (1: 1000 v/v). The test temperature is set at 37°C. Measurements were performed in triplicate.

2.6. FTIR
FTIR of artemisinin crystal, SEDDS of artemisinin, and the formulation without artemisinin were determined. Artemisinin crystal and SEDDS were mixed with potassium bromide and analyzed by FTIR spectrophotometer. The scanning range was 450–4000 cm⁻¹.

2.7. In vitro dissolution study
The dissolution tests were carried out using the Copley Scientific Dis-6000 type 1 dissolution apparatus. The SEDDS formulation and artemisinin crystal with the equivalent weight of artemisinin (14 mg) were put into a hard gelatin capsule size 0 and placed in the dissolution tube. The dissolution tubes were filled with pH 6.8 phosphate buffer as a medium. The tests were carried out at 37°C ± 0.5°C with a stirring speed of 100 rpm. Each 5 mL of samples were taken and replaced with 5 mL of new medium every 15, 30 45, 60, 90, 120, and 150 minutes. 1 mL of samples was diluted with ethanol and 0.2% sodium hydroxide (1:4 v/v) in volumetric flask. The solutions were heated at 50°C for 30 minutes in a water bath. The absorbance of the samples were then measured using UV-VIS Spectrophotometer Agilent Technology's Cary 60 at a wavelength of 290 nm.

3. Results and Discussion

3.1. Physical stability of Artemisinin Self Emulsifying Drug Delivery System after emulsification
The physical stability of the resulting emulsion is an important parameter for maintaining the drug dissolved concentration. Instability of emulsion can lead to precipitation of the supersaturated drug before being absorbed in the intestine. Physical stability of all formulations was observed visually every 15 minutes for 6 hours. The presence of any separation phase in emulsions like creaming, flocculation, and precipitation was observed. A stable emulsion is an emulsion system where the droplets remain uniformly distributed throughout the continuous phase for a certain time [17]. Emulsion instability is often indicated by the appearance of flocculation or creaming. Flocculation occurs when the droplets aggregate and without fully coalescing. The disperse phase (oil) of poor
stability emulsion is less dense and rises to the top to form a layer of more concentrated emulsion and it is indicated as creaming [17]. Table 2. shows the Physical appearance, flocculation times, and creaming times of the resulting emulsions of SEDDS formulation. Flocculation times were measured when at least one floc appeared in the emulsion and creaming times were measured when creaming first appeared on top of the emulsion. The result indicated that the formulation with higher surfactant concentration (formulation 6-10) tend to be more stable than the formulation with the lower surfactant concentration (formulation 1-5). The formulation with 60% surfactant concentration had no flocculation and creaming observed for 6 hours. This might be because the increased of interface film strength in the higher surfactant concentration. High surfactant concentration can also form thick multilayer absorption and lower interfacial tension between dispersed oil and continuous phase that increases the stability of the emulsion system [18-19]. As we can see in Table 2, flocculation occurred more rapidly as the co-surfactant concentration decreased. It is indicating that PEG 400 as a co-surfactant can slow down the flocculation. PEG 400 is a polymer that can stabilize the emulsion by enhance the viscosity of water and decrease the aggregation of oil droplets [18].

### Table 2. Physical appearance, flocculation times, and creaming times of emulsion.

| Samples | Physical appearance | Flocculation times (minutes) | Creaming times (minutes) |
|---------|---------------------|-----------------------------|--------------------------|
| F1      | Cloudy white        | 30                          | 240                      |
| F2      | Cloudy white        | 30                          | 240                      |
| F3      | Cloudy white        | 45                          | 240                      |
| F4      | Cloudy white        | 105                         | 240                      |
| F5      | Cloudy white        | 255                         | 300                      |
| F6      | White transparent   | -                           | -                        |
| F7      | White transparent   | -                           | -                        |
| F8      | White transparent   | -                           | -                        |
| F9      | Transparent         | -                           | -                        |
| F10     | Transparent         | -                           | -                        |

#### 3.2. Droplet size and polydispersity of emulsions

The droplet size of the resulting emulsion of SEDDS has a major effect on the dissolution and bioavailability of the drug. As seen in Table 3, the most stable formulations from physical stability observation have droplet sizes in the range of 114,17 – 247,93 nm. It has been reported that the size of artemisinin pure crystal was 5021,7 nm [20]. It is indicating the reduction of artemisinin particle size from 5021,7 nm to dispersed emulsion droplets in the range of 114,17-247,93 nm. The reduction of particle size could impact the dissolution rate according to Noyes–Whitney equation [21]. The reduction of droplet size also has been reported to increase the bioavailability of drugs. It can be seen in Table 3, the droplet size was decreased as the concentration of PEG 400 increased. The addition of PEG 400 can lower the interfacial tension and lead to produce smaller particle size. It also might be contributed to the stability of the emulsion system since the Brownian motion of smaller particle size could inhibit the flocculation [18].

The polydispersity index is a representation of the distribution of the size population. The numerical value of the polydispersity index range from 0.0 which indicates a perfectly uniform sample to 1.0 which indicates a very high polydispersity sample [22]. The polydispersity index bigger than 0.7 has a broad particle size distribution and it may not suitable for the dynamic light scattering method [22]. The polydispersity index of the emulsions was in the range of 0.35-0.56 which all below 0.7 and not considered to be a broad particle size distribution and acceptable to use dynamic light scattering method.
Table 3. Droplet Size and Polydispersity Index of Emulsion

| Samples | Droplet Size | Polydispersity Index |
|---------|--------------|----------------------|
| F6      | 247.93 ± 2.97| 0.56 ± 0.01          |
| F7      | 226.47 ± 1.30| 0.43 ± 0.05          |
| F8      | 151.53 ± 1.21| 0.39 ± 0.02          |
| F9      | 120.80 ± 0.10| 0.35 ± 0.01          |
| F10     | 114.17 ± 1.63| 0.44 ± 0.01          |

3.3. Zeta Potential

The stability of SEDDS emulsion can be monitored by measuring zeta potential. Extremely positive or negative zeta potential values cause higher repulsive forces. The repulsion between particles will prevent aggregation of each particle and makes droplets easier to redispersion [23]. Zeta potential values of the selected formulations were found in the range of -23.23 - -2.33 mV (Table 4). The high values of zeta potential (more than 20 mV) provide system stability and are less prone to form aggregates. The negative zeta-potential value can be due to the presence of negatively charged carboxyl groups in fatty acid [24-25].

Table 4. Zeta Potential of Emulsion

| Samples | Zeta Potential |
|---------|----------------|
| F6      | -2.33          |
| F7      | -5.53          |
| F8      | -21.23         |
| F9      | -23.23         |
| F10     | -15.00         |

3.4. FTIR

![Figure 1. FTIR spectra of artemisinin, the formulation of SEDDS without artemisinin, and artemisinin pure crystal.](image)
Figure 1 displays the FTIR spectra of SEDDS of artemisinin, the formulation of SEDDS without artemisinin, and artemisinin pure crystal. As can be seen in Figure 1, FTIR spectra of pure artemisinin showed CH stretching at 2926 cm\(^{-1}\), C=O stretching in δ lactone at 1735 cm\(^{-1}\), CH\(_3\) stretching at 1369 cm\(^{-1}\), and O-O stretching at 856 cm\(^{-1}\). The SEDDS FTIR spectra also showed the CH stretching at 2927 cm\(^{-1}\), C=O stretching in δ lactone at 1728 cm\(^{-1}\), CH\(_3\) stretching at 1354 cm\(^{-1}\), and O-O stretching at 852 cm\(^{-1}\). The FTIR spectra of SEDDS of artemisinin showed all the peaks of artemisinin and all the peaks of the other ingredients (formulation without artemisinin) without any significant shift and the presence of new peaks. Thus, indicating the presence of artemisinin in the formulation and there was no change in the functional groups of the drug and each ingredient.

3.5. *In vitro Dissolution*

In vitro dissolution testing or in vitro release testing is a parameter that is important to the development of the pharmaceutical drug. In vitro dissolution testing is an analytical method to measure the drug release in liquid media. The liquid media used was pH 6.8 phosphate buffer that was a representation of the physiological small intestine fluid. Figure 2 displays that the drug release profile of SEDDS of artemisinin and artemisinin pure crystal in pH 6.8 phosphate buffer showed that the drug release profile of SEDDS of artemisinin was found to be significantly higher compared to artemisinin pure crystal. The SEDDS of artemisinin released the drug up to 98.6\% in 150 minutes and the artemisinin crystal released the drug up to 8.67\% in 150 minutes. It ensures that SEDDS of artemisinin can improve the in vitro dissolution profile of artemisinin.

![Figure 2. Dissolution curve of SEDDS of artemisinin and artemisinin pure crystal](image)
4. Conclusions
This research work determined the droplet size, polydispersity index, zeta potential, and functional group of the selected formulation of artemisinin SEDDS, then evaluated the optimum formulation of SEDDS and the artemisinin pure crystal using in vitro dissolution test. The droplet size of the selected formulation was in the range of 114.17-247.93 nm, polydispersity index was 0.35-0.56, and zeta potential value was -23.23 - -2.33 mV. The FTIR spectra showed the presence of artemisinin in the formulation without any change in the functional group of each ingredient. The in vitro dissolution test showed a significant increase of dissolution profile compared to artemisinin pure crystal. Thus, ensuring the SEDDS formulation for antimalarial drug artemisinin is a promising method to improve the dissolution profile of the drug.

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