Preparation of Curcumin Nanoemulsion in Soybean Oil – Tween 80 System by Wet Ball Milling Method

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Abstract. Curcumin nanoemulsion which has a particle size of 20-200 nm, is one of the curcumin drug delivery system that can increase curcumin solubility and bioavailability. The previous method of curcumin nanoemulsion formulation is modified thin-film hydration followed by sonication that has a long process and using organic solvent. This study aims to make curcumin nanoemulsion by wet ball milling method in soybean oil – Tween 80 system. Nanoemulsion is expected to have smallest particle size, high curcumin loading capacity and good stability during storage. Preparation of curcumin nanoemulsion is done by wet milling method which consists of curcumin as drug, soybean oil as curcumin solvent, tween 80 as stabilizer, water as dispersed medium and milling beads as milling media on the vial with various milling time. Particle size and polydispersity index are monitored using dynamic light scattering while nanoemulsion morphology is observed using digital imaging microscope. Results show that optimum milling times is 24 h and the highest curcumin added which produces less than 500 nm particles is 300 mg which is stable during 60 days of storage. Particle size of the nanoemulsion is ranging from 127 – 338 nm. In addition, wet ball milling methods is relatively simple and easy to apply.

1. Introduction
Indonesia is one of the countries that has various plant as a potential drug. One of the plant is turmeric [1]. Based on Badan Pusat Statistik [2], production of turmeric in Indonesia is 128.682 ton each year with the harvested area of 64.967.310 m². This makes turmeric becomes one of the most abundant plant in Indonesia. Turmeric contains curcumin, a compound which provides effect on health [3]. Curcumin is mainly polyphenolic compound that is obtained from turmeric rhizome’s [4]. Curcumin potentially developed as an antidiabetic, anti-inflammation and antioxidant agent [5]. Nevertheless, curcumin has low solubility and bioavailability in water and unstable in storage which make curcumin application as drug is limited. Therefore, curcumin is designed as nanoemulsion drug delivery system [6].

Nanoemulsion is a colloidal system, oil dispersed in water, with an average diameter between 20-200 nm. Nanoemulsion can increase the solubility and bioavailability of a drug because of its relatively small size which enhances the distribution of drugs in the body. In the formation of nanoemulsion, oil and water are required [7]. The oil used in this research is soybean oil which has capacity to solubilize 2.4 mg of curcumin per mL [8]. To stabilize nanoemulsion, a stabilizer (surfactant) is required to prevent...
aggregation of nanoemulsion [7]. The surfactant used in this research is Tween 80, which is a type of non-ionic surfactant and generally recognized as safe surfactant which is non-toxic and non-irritating chemicals [10].

Various studies have been carried out in the preparation of curcumin nanoemulsions. Anuchapreeda et al. [8], proposes research on the preparation of lipid nanoemulsions combined with curcumin for cancer therapy. The method used is the modified thin film hydration followed by sonication. In that study, nanoemulsion was produced with an average particle diameter of 47-55 nm. Preparation of nanoemulsion with modified thin film hydration has a long procedure and uses harmful organic solvents. Therefore, preparation of nanoemulsion in this study uses wet ball milling method, a simple and straight forward method [11]. Wet ball milling method is the process of making particles smaller than the size of the original material by continuously milling the particles using milling beads. The longer the milling time used, the smaller the particle size. Nevertheless, at a certain time the particle size will enlarge due to friction that occurs between particles which indicates the stability of nanoemulsion [12]. Therefore, the optimum milling time and stability of nanoemulsion are very important to be determined in the study.

Based on the description and previous research above, this research is conducted in preparation of curcumin nanoemulsion in the soybean oil - Tween 80 system by wet ball milling method. This research is aimed to obtain curcumin nanoemulsion with small particle size and stable during the storage time.

2. Experimental

2.1. Apparatus and materials

Apparatus that were used is 50 mL beakers, 100-1000 µL micropipettes (Vitlab), tips, magnetic stirrers, glass stirrers, stirrer plates, milling beads, aluminum foil, vial bottles, eppendorf tubes, Vortexer Cleaver (Scientific Ltd.), Delsa™ Nano C Particle Analyzer (Beckman Coulter), Digital Imaging Microscope (Olympus BX51).

Materials that was used is curcumin and Tween 80 were purchased from Sigma-Aldrich, soybean oil, purified water, methanol, ethanol 96%.

2.2. Preparation of Curcumin Nanoemulsion

Pre-emulsion solution was made by mixing 1 mg curcumin, 62.5 mg Tween 80, 200 µL soybean oil, and 5 mL purified water in beaker glass. Following this step, 2.5 mL milling beads and 1 magnetic stirrer were added to the vial bottle. Pre-emulsion solution then added to the vial bottle. The solution was milled above the stirring plate with various milling time i.e 1, 4, 8 and 24 h. During every determined milling time, 200-300 µL curcumin nanoemulsion was taken from vial bottle and saved in the Eppendorf tube. Next, the Eppendorf tubes were saved at 4°C and at room temperature 25°C and further analyzed to measure the particle size and polydispersity index. The same procedure was carried out in making variations of curcumin loading with a mass of 100, 200, 300, 400, and 500 mg. In the milling process, optimum milling time was applied.

2.3. Measurement of Particle Size and Polydispersity Index

Particle size and polydispersity index were determined using Delsa™ Nano C Particle Analyzer Beckman Coulter instrument, using the principle of dynamic light scattering. Measurements was made using distilled water at 25°C, refractive index 1.3328, viscosity 0.8878, dielectric constant 78.3 and in disposable cells. At each measurement, 50 µL of curcumin nanoemulsion solution is dissolved in 2450 µL of purified water. Previously, an optimization method had been performed to obtain a comparison of the curcumin nanoemulsion composition with the solvent used.

Nanoemulsion stability test as a function of time was carried out by comparing the particle size and polydispersity index between nanoemulsion at day 1 and nanoemulsion stored until day 60. The effect of storage method at room temperature and at 4°C were also determined by comparing the same parameters as previously mentioned.
2.4. Nanoemulsion Morphology

The morphology of curcumin nanoemulsion was recorded using Digital Imaging Microscope Olympus BX51. The magnification used was 400 times.

3. Results and Discussion

Preparation of curcumin nanoemulsion in this research is carried out using wet ball milling method by mixing all drug nanoemulsion forming materials consist of oil, water, curcumin, and milling media into a vessel. Milling process is carried out continuously at various milling time i.e 1, 4, 8, and 24 h. The sample then being analyzed for its nanoemulsion size, polydispersity index, morphology, and stability.

Nanoemulsion size and polydispersity index are measured using Delsa™ Nano C Particle Analyzer with a dynamic light scattering principle. Both nanoemulsion size and polydispersity index are dependent on milling time variation. Nanoemulsion size indicates the average diameter of nanoemulsion in the system which decreases while milling time increases (Figure 1). Our results show that particle size is linearly reduced as the milling time increases. At 1 h, the particle size is 533 nm and reduced to 127 nm at 24 h milling time. Therefore at 24 h milling time, curcumin nanoemulsion has the smallest size than the shorter milling time. This indicates that the rate of nanoemulsion size reduction obeys first-order kinetic theory.

Polydispersity index indicates the dispersity and homogeneity of nanoemulsion size which is ranging from 0 to 1. A homogenous and well-dispersed colloidal system has polydispersity index lower than or approaching 0,1. Figure 2 shows polydispersity index in which at 1, 4, and 8 h milling time the value of polydispersity index is almost identical (0.30). At 24 h milling time, polydispersity index produces smaller value than the previous results which is 0.15. Therefore 24 h milling time provides the best colloidal system of curcumin nanoemulsion.

To further confirm the results, nanoemulsion morphology is analyzed using Digital Imaging Microscope. The images obtained support nanoparticle size and polydispersity index results where it can be seen that the particle size is reduced. Figure 3 (a-c) indicates that the particle size decreasing slightly at 1,4 and 8 h milling time while 24 h milling time image (Figure 3 d) signifies the smallest particle size.
Figure 3. Morphology of curcumin nanoemulsion at various milling time. (a) 1 h, (b) 4 h (c) 8 h (d) 24 h.

Table 1. Stability of curcumin nanoemulsion particle size toward milling time.

| No | Milling Time (h) | Nanoemulsion Size (nm) |
|----|------------------|------------------------|
|    |                  | Day 1 | Day 60, 4°C | Day 60, 25°C |
| 1  | 1                | 532.7 | 985.6 | 637.7 |
| 2  | 4                | 443.2 | 398.5 | 491.6 |
| 3  | 8                | 325   | 1096.3 | 273.8 |
| 4  | 24               | 126.7 | 120   | 120   |

Table 2. Stability of polydispersity index toward milling time.

| No | Milling Time (h) | Polydispersity Index |
|----|------------------|----------------------|
|    |                  | Day 1 | Day 60, 4°C | Day 60, 25°C |
| 1  | 1                | 0.297 | 0.363 | 0.32 |
| 2  | 4                | 0.305 | 0.319 | 0.326 |
| 3  | 8                | 0.296 | 0.429 | 0.299 |
| 4  | 24               | 0.147 | 0.101 | 0.151 |

To monitor the stability of 1 mg curcumin nanoemulsion, particle size and polydispersity index are observed as temperature (4°C (fridge temperature) and 25°C (room temperature) and storage time function (60 days). Both temperature and storage time affect the change of nanoemulsion size and polydispersity index because it causes coalescence at the nanoemulsion system. At 24 h milling time, nanoemulsion size and polydispersity index are relatively identical between day 1 and day 60 observation (Table 1 and Table 2). Meanwhile, at 1, 4, and 8 h milling time both nanoemulsion size and polydispersity index are fluctuated randomly. This implies that larger nanoemulsion size and polydispersity index at day 1 influence the stability of nanoemulsion. It is possible that the repulsion between particles is minimum which facilitates coalescence during storage. On the other hand, small particle size and polydispersity index produce higher repulsion effect which hinders the coalescence.
between particle. The results signify that curcumin nanoemulsion is stable at 24 h milling time during 60 days storage both at 4°C and 25°C.

To further improve the amount of curcumin embedded in soybean oil droplets while maintaining the size of the particles, more curcumin is added in the system. Figure 4 and 5 show the particle size and polydispersity index of curcumin nanoemulsion when various curcumin amount (100, 200, 300, 400 and 500 mg) are incorporated in the system.

![Figure 4. Effect of curcumin loading to nanoemulsion size at optimum milling time.](image1)

![Figure 5. Effect of curcumin loading to polydispersity index at optimum milling time.](image2)

The addition of 100, 200 and 300 mg produces particle size ranging from 255 – 338 nm which is still classified as particle size in the nanoemulsion system. Meanwhile, the addition of 400 and 500 mg of curcumin dramatically change the particle size to more than 3000 nm which is classified as particle size in the conventional emulsion. Polydispersity index at 100, 200, 300 and 400 mg are relatively the same at around 0.2 and dramatically increases at 500 mg. Thus, the maximum curcumin that can be added while maintaining nanoemulsion size is 300 mg.

4. Conclusion
Curcumin that was designed as nanoemulsion using wet ball milling method can improve the solubility of curcumin in water. Optimum milling time in the preparation of curcumin nanoemulsion is 24 h. On the optimum milling time, curcumin nanoemulsion is stable at 4°C and 25°C during 60 days storage time. The maximum curcumin that can be added to form nanoemulsion is 300 mg.

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