Effect of High Speed Homogenizer Speed on Particle Size of Polylactic Acid

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Abstract. Polylactic acid (PLA) nanoparticles could provide extended release of drugs, reducing the frequency of injection and increasing the compliance diabetic retinopathy patients. This requires nanoparticles with a mean diameter of approximately 200 nm to facilitate penetration of small and narrow blood vessels in the retina. Mean diameter of PLA particles prepared using the emulsification-evaporation method was found to depend linearly on the speed of the high speed homogenizer, based on the data taken in the range of 1,000-10,000 rpm. PLA nanoparticles with a mean particle diameter of 190 nm with a spherical morphology could be obtained using a homogenizer speed of 10,000 rpm, allowing the use of PLA nanoparticles for extended release of drugs in the retina.

Keywords: Particle size; polylactic acid; high speed homogenizer

1. Introduction
Diabetes mellitus is the metabolic disease characterized by chronic hyperglycemia where impaired metabolism of carbohydrate, fat, and protein due to insufficient amount or damaged insulin, or both [1]. In Indonesia, patients with diabetes mellitus increased from 1.1% in 2007 to 2.1% in 2013 [2]. Diabetes mellitus that is not properly treated could lead to diabetic retinopathy, a retinal disorder in the posterior part of the eye where damaged and blocked blood vessels could cause blindness. There are obstacles in drug delivery to the posterior part of the eye related to the anatomy and physiology of the eye. The intraocular drug delivery method (injection) overcomes blood-retinal barrier and allows intravitreal or intraretinal high drug concentration. This invasive approach involves repeated penetration of the eyeball with complications related to injection, such as, increasing intraocular pressure and retinal hemorrhage.

Treatment of diabetic retinopathy requires an extended drug delivery system to the posterior part of the eye. This could be achieved through extended release of drugs encapsulated in nanoparticles made of biodegradable polymer such as polylactic acid (PLA), an FDA-approved biodegradable polymer obtained from the esterification of lactic acid [3]. This approach would reduce the injection frequency, hence, improving patient compliance and reducing the possibility of complications related to the injection. Effective inclusion of nanoparticles in the ocular veins could be achieved if the diameter of the nanoparticles is less than 200 nm. This small size is also required to avoid aggregate formation that can cause an embolism in the retina veins [4].

Emulsion-solvent evaporation, the method that has been commonly used to prepare nanoparticles. In this method, oil-in-water nanoemulsion is formed by stirring the mixture of organic solution that contains polymers and the aqueous phase, followed by evaporation of the
organic solvent [5]. To prepare nanoparticles, stirring method that is commonly used are the high pressure homogenization, high speed homogenization, or ultrasonication. A high speed homogenizer is used in this study because a high pressure homogenizer is much expensive, while the high energy input in ultrasonication could cause hot spots that degrade drugs. In a high speed homogenizer, nanoemulsion is formed through strong shear stress and formation of turbulence in the rotor-stator assembly. Various factors affect the size of nanoparticles, including the molecular weight of the polymer, oil to water mass ratio, surfactant and organic solvent used, and stirring speed. The objective of this study is to determine the effect of stirring speed on the morphology and the particle size distribution of the nanoparticles produced, in particular those with mean diameter of around 200 nm.

2. Methods
The organic phase was formed by dissolving 200 mg PLA (acid terminated, MW 55-85 kDa) into 7.5 mL chloroform. After PLA was completely dissolved, 3.75 mL acetone was added to the solution. The aqueous phase was formed by dissolving 2 g PVA into 100 mL of deionized water by mixing at 500 rpm and heating at 90 °C for 1 h, followed by cooling the PVA solution to room temperature. To form an oil-in-water emulsion the organic phase was added into the aqueous phase, and homogenized at the speed level of 1000, 4000, 7000, and 10,000 rpm for 10 min using an IKA T-25 high speed homogenizer. The mixture is cooled to prevent excessive heating. After homogenization process, the solution was then stirred at a low speed at room temperature for 15 hours to evaporate the organic solvents. The solution was then separated using a microcentrifuge operated at a speed of 13,000 rpm for 6 min, the resulting particles washed with demineralized water to remove the remaining surfactant, and freeze dried.

3. Results and Discussion
Figure 1 shows the scanning electron microscope (SEM) images of PLA nanoparticles prepared using a high speed homogenizer operated at a speed of 1,000, 4,000, 7,000, and 10,000 rpm.
Figure 1. SEM images of PLA nanoparticles prepared with a homogenizer speed and magnification of (a) 1,000 rpm (500x), (b) 4,000 rpm (500x), (c) 7,000 rpm (500x), (d) 10,000 rpm (1000x).

All of the SEM images show spherical morphology and higher homogenization speed produce smaller size PLA particles. At higher homogenization speeds, higher energy density is exerted on the solution that directly reduce the emulsion droplet size. Shear stress is also inversely proportional to emulsion droplet size. Increasing of shear stress will reduce droplet size to produce nanodroplets. Then, the nanodroplets became nanoparticles as organic solvent diffuse through the solution and evaporate into the atmosphere.

The mean diameter of the particles obtained were determined using a Zetasizer particle size analyzer, based on the intensity of the light scattered by the dispersed particles. The data given in Table 1 and shown in Figure 2 show decreasing particle size as the homogenizer speed increases, consistent with the SEM results. Increasing the speed of the high speed homogenizer also produced a sharper particle size distribution as indicated by the decreasing standard deviation of the mean. At a homogenizer speed of 10,000 rpm, particles with the smallest mean diameter of 190 nm could be produced, suitable for extended release of drugs in the retina of diabetic retinopathy patients. As a comparison, Jose et al. [6] used a sonicator and obtained agglomerated nanoparticles as shown in Figure 3.

Table 1. Mean particle diameter as a function of homogenizer speed of 1,000-10,000 rpm.

| Speed (rpm) | Mean particle diameter (nm) | Standard deviation (nm) |
|-------------|-----------------------------|-------------------------|
| 1,000       | 2,010                       | 113                     |
| 4,000       | 1,153                       | 47                      |
| 7,000       | 788                         | 24                      |
| 10,000      | 190                         | 16                      |

The following regression equation gives a linear dependence of particle diameter on the high speed homogenizer ($r^2$ equal to 0.975):

$$ d (\text{nm}) = 2100 - \text{speed (rpm)}/5 $$  \hspace{1cm} (1)

The proposed equation could be used only if the emulsion is an oil-in-water type having organic solvent to water ratio of 7.5:100 (v/v) and 2% (w/v) surfactant solution (PVA).
4. Conclusions
Mean diameter of PLA particles prepared using the emulsification-evaporation method was found to depend linearly on the speed of the high speed homogenizer, based on the data taken in the range of 1,000-10,000 rpm. PLA nanoparticles with a mean particle diameter of 190 nm (standard deviation of 16 nm) and a spherical morphology could be obtained using a homogenizer speed of 10,000 rpm, allowing use of the nanoparticles for extended release of drug in the retina.

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