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Development of size-tunable polymeric nanoparticles for drug delivery applications

Komkrich Sawasdee [1], Ployphailin Choksawad [1], Sopida Pimcharoen [2], Kanlaya Prapainop[1,*]

[1] Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok, Thailand.
[2] Samsenwittayalai School, Rama VI Road, Bangkok, Thailand.

*Corresponding author. Email: kanlaya.pra@mahidol.edu

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ABSTRACT

Background: Poly lactide-*co*-glycolide (PLGA) nanoparticles (NPs) have been widely used in drug delivery applications because of their excellent properties such as biocompatibility, and biodegradability along with the ability to deliver hydrophobic drugs, increase drug bioavailability and improve drug absorption to targeted cells in both oral and parenteral administrations. The PLGA NPs can be synthesized using an emulsion solvent evaporation method. Each parameter during synthesis plays a role in the formation of nanoparticles and can affect NP size; important factors for successful development of a drug delivery system.
Aims: The aim of this study was to prepare different sizes of PLGA NPs by investigation of four factors (molecular weight, MW) of PLGA, emulsifier concentrations, organic solvent type and power of ultrasonication) involved in PLGA nanoparticle synthesis.
Methods: PLGA nanoparticles were prepared by an emulsion solvent evaporation method. Size and size distribution were analyzed by dynamic light scattering and polydispersity index (PdI).
Results: The effect of four parameters: PLGA MW, emulsifier concentrations, solvent types, and amplitude of ultrasonication on PLGA NPs preparation were evaluated. Changing one parameter results in different sizes of PLGA NPs that varied from 150-300 nm. PdI which is an indicator for determination of size distribution of NPs, varied with an overall value <0.2.
Conclusion: MW of PLGA polymer, emulsifier concentration, type of organic solvent and power of ultrasonication affect the size and size distribution of PLGA NPs.

INTRODUCTION

Cancer is a worldwide health problem with millions of people suffering from this disease in both economically developed countries and developing countries [1]. The current strategies for treatment of cancer include surgery, radiation, immunotherapy or chemotherapy. The chemotherapeutic drug is administered intravenously can spread to accumulate in off-targeted tissues or organs leading to undesirable side effects. To solve these problems, anti-cancer drug loaded nanoparticles have gained extensive attention to use as an effective drug delivery system to specific target sites [1, 2].

Polymeric NPs have unique properties including stability, biocompatibility and ease of surface chemistry modification [3, 4]. Therefore these nanoparticles have been used in drug delivery

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systems to protect drugs from bio- and chemical degradation which also help to increase drug stability. In addition, the nanoparticle surface can be further modified for specific targeting purposes. For example, NPs are conjugated with specific antibodies against cancer cells and used to deliver anti-cancer drugs for therapeutic purpose [5]. Poly lactide-*co*-glycolide (PLGA) is one of the polymers that can form nanoparticles [6]. This linear copolymer can be hydrolyzed into the two non-toxic monomers: lactic acid and glycolic acid [7], therefore, PLGA has been approved for use in biomedical applications such as drug delivery by the United States Food and Drug Administration (FDA) and European Medicine Agency (EMA) [8, 9]. In order to develop a successful drug carrier system, the size of NPs is a key factor especially in cancer therapy [1, 2].

Several reports show that size and size distribution of NPs play important role in term of *in vivo* biodistribution, biological fate and toxicity [10]. Smaller particles have relatively higher uptake efficiency to the targeted cells compared to larger particles but have smaller inner space for drug entrapment [11]. In addition, the drugs in the small NPs are at or near the particle surface, leading to faster drug release compared to large particle size [12]. This faster release rate in smaller nanoparticles was also observed in a bovine serum albumin protein release model [13]. Therefore, the small NPs would be suitable for drug delivery that requires fast release rate whereas the large particles would be appropriate for drug that requires slow release rate. Due to the importance of drug carrier size, the development of synthesis methods for preparation of different NP sizes is important to provide the most optimal size and property to each specific drug delivery requirement [14].

This study focuses on development of tunable size of PLGA NPs. Four different parameters that might affect particle sizes (molecular weight of PLGA polymer, emulsifier concentrations, type of organic solvent and power of ultrasonication) are investigated. The overall scheme of PLGA NPs synthesis is shown in Figure 1.

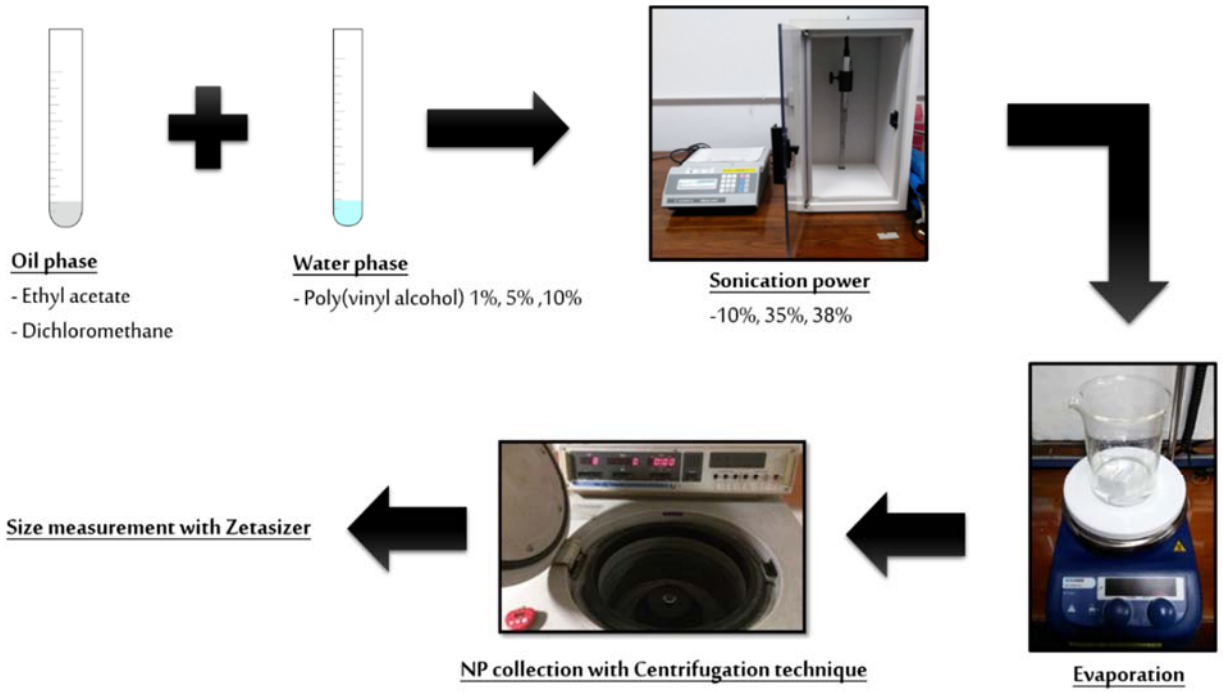

Figure 1. Schematic representation of general procedure of preparation of PLGA nanoparticle by emulsion solvent evaporation method.

METHODS

Materials

PLGA NPs were prepared by a modification of emulsion solvent evaporation method as previously described [15,16]. Four parameters were investigated: (I) size of PLGA polymer (60,000 g/mol and 80,000 g/mol), (II) concentration of PVA that acts as emulsifier (1-10 % (w/v)), (III) type of organic solvent (EtOAc and DCM) and (IV) power of ultrasonication (10%, 35% and 38% amplitude).

Preparation of PLGA NPs

PLGA NPs were prepared by a modification of emulsion solvent evaporation method as previously described [15, 16]. Four parameters were investigated: (I) size of PLGA polymer (60,000 g/mol and 80,000 g/mol), (II) concentration of PVA that acts as emulsifier (1-10 % (w/v)), (III) type of organic solvent (EtOAc and DCM) and (IV) power of ultrasonication (10%, 35% and 38% amplitude).

Preparation of PLGA NPs using different molecular weight of PLGA

A total of 50 mg of two different MW of PLGA polymer (60,000 and 80,000 g/mol) was dissolved in 0.5 mL EtOAc. The PLGA solution was added dropwise to 2 mL of PVA dissolved in water under vortex. Afterwards, the emulsion solution was treated with 3 cycles of 30 seconds pulse (38% amplitude). At the end of the cycles, the solution was quickly added into 25 ml of 0.3 % PVA under stirring for 4 hours to evaporate the organic solvent. The PLGA nanoparticles were collected by centrifugation at 15,000 rpm for 15 minutes. After that, PLGA nanoparticles were washed for three times with deionized water and followed by lyophilization.

Preparation of PLGA NPs using different emulsifier concentrations

To study the effect of emulsifier concentrations, 50 mg PLGA polymer (60,000 g/mol) was dissolved in 0.5 mL EtoAc. The PLGA solution was added dropwise to 2 mL of different PVA concentrations dissolved in water (1%, 5% and 10% (w/v)) under vortex. The mixture was emulsified, collected and stored using the same procedure as described above.

Preparation of PLGA NPs using different organic solvent types

To study the effect of organic solvent type, 50 mg PLGA polymer (60,000 g/mol) was dissolved in 0.5 mL of different organic solvents (EtOAc or DCM). The PLGA solution was added dropwise to 2 mL of 5% (w/v) PVA dissolved in water under vortex. The mixture was emulsified, collected and stored using the same procedure as described above.

Preparation of PLGA NPs using different power of ultrasonication

To study the last parameter: the power of ultrasonication, 50 mg PLGA polymer (60,000 g/mol) was dissolved in 0.5 mL EtOAc. The PLGA solution was added dropwise to 2 mL of 5% (w/v) PVA dissolved in water under vortex. The mixed solution was subsequently emulsified by sonication with 3 cycles of 30-second pulses (10%, 35% and 38% amplitude). The mixture was collected and stored using the same procedure as described above.

Characterization of particle size and PdI value

NPs were dispersed in Milli Q water at concentration of 0.1 mg/ml before characterization. The size and PdI value of the particles were analyzed using Nanosizer 90 ZS (Malvern Instrument Ltd., Malvern, UK) at 25^{o}C. All measurements were carried out as the average of value with triplicate measurements and at least 10 runs within each analysis. The results were reported as a mean of three independent readings ± standard deviation.

RESULTS

PLGA NP size and size distribution from different MW of PLGA

In this experiment, the particles prepared by 60,000 and 80,000 g/mol PLGA have a size about 179 and 261 nm respectively (Figure 2). Both NPs prepared by the two MW showed PdI values less

than 0.2 which indicated that the NPs were monodispersed.

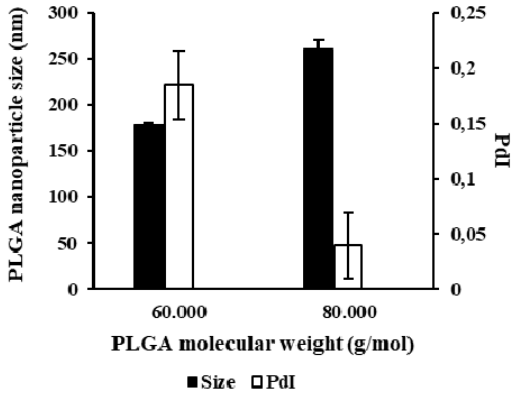

Figure 2. Characterization of PLGA NPs prepared using different molecular weight of PLGA. The 5% PVA, EtOAc was applied to the reaction and 38% power of sonication was used. Size (black bars, left y-axis) and PdI (white bars, right y-axis) values are presented.

PLGA NP size and size distribution from different emulsifier concentrations and organic solvent types

The sizes of PLGA nanoparticles decreased when the PVA concentration was increased (Figure 3). Using EtOAc as solvent (Figure 3A), at 1% (w/v) PVA, the particle size was about 259 nm. When the PVA concentration was increased to 5% and 10% (w/v), the particle sizes were 179 and 176 nm, respectively. It was found that at 5% and 10% (w/v) PVA the particle sizes were not significantly different. The PdI value that indicates nanoparticle size distribution showed the value less than 0.2 which suggested that PLGA nanoparticles prepared by these PVA concentrations were monodispersed. Similar results were also obtained from NPs preparation using DCM as a solvent (Figure 3B).

NPs size and PdI values prepared in EtOAc were smaller (170-220 nm) (Figure 3A) than the particles that prepared in DCM (300-500 nm) (Figure 3B).

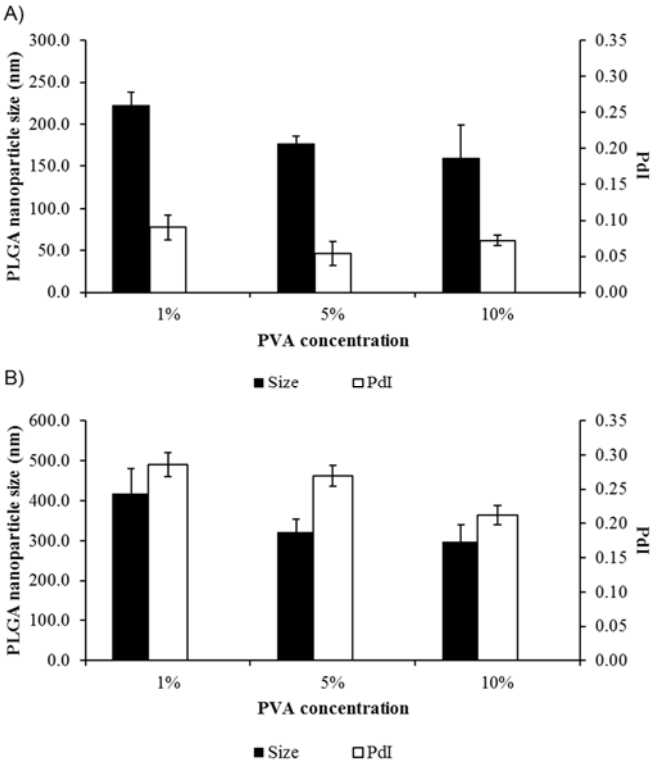

Figure 3. Characterization of PLGA NPs prepared using different PVA concentrations and different solvents. A) NPs prepared using EtOAc, B) NPs prepared using DCM. The 60,000 g/mol PLGA, and solvent were applied to the reaction and 38% power of sonication was used. Size (black bar, left y-axis) and PdI (white bars, right y-axis) values are presented.

PLGA NP size and size distribution from different power of ultrasonication

NPs prepared by 38% sonication were of smaller size (179 nm) than particles prepared by 10% (246 nm). This result indicated that higher power of sonication induced the production of smaller size of NPs. The PdI values from both conditions were less than 0.2 that represented monodispersity of NPs (Figure 4).

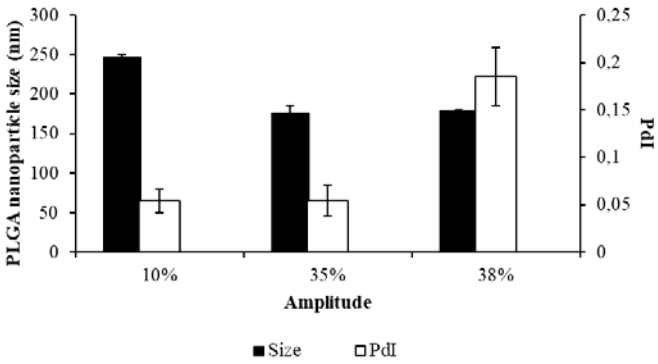

Figure 4. Characterization of PLGA NPs prepared by using different ultrasonication power. The 5% PVA, 60,000 g/mol PLGA and EtOAc were applied to the reaction. Size (black bars, left y-axis) and PdI (white bars, right y-axis) values are presented.

PLGA NP size after long storage in aqueous solution

To use NPs as a drug delivery system the stability of drug carrier is important. Figure 5 illustrates examples of NPs size after long storage in aqueous solution up to 7 months. The NPs size remains similar to short storage time and no sign of aggregation was observed. This result suggested that the obtained PLGA NPs are stable up to 7 months and has high potential for development as a drug carrier system.

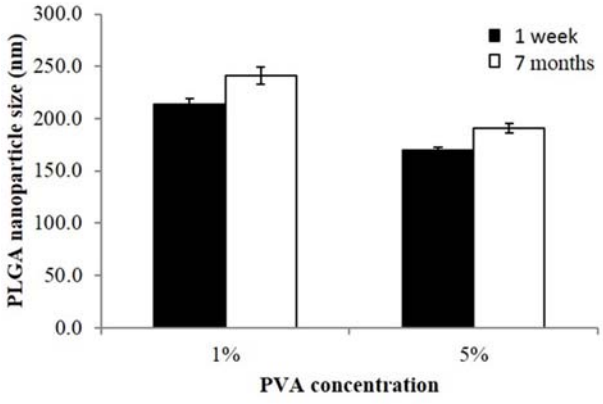

Figure 5. Characterization of PLGA NPs prepared by using different PVA concentrations and after long term storage. NPs prepared using 60,000 g/mol PLGA, and EtOAc were applied to the reaction and 35% power of sonication was used. Size after one week (black bars) and size after 7 months (white bars) are presented.

DISCUSSION

PLGA NP size is easily tunable by simple changes in the synthesis parameters. With high MW of PLGA a larger particle size is produced because the long chain of polymer can induce larger oil droplets [17]. Data show that PVA can be used to stabilize emulsion and lead to formation of small and monodispersed particles [18, 19]. Therefore, when increasing the concentration of PVA the size of particles tend to decrease. In addition, organic solvent can affect the particles size of PLGA NPs. The NPs prepared by DCM were bigger than particles prepared by EtOAc. These results could be due to different properties of the solvent. DCM is a non-miscible solvent in water, unlike EtOAc. Therefore, DCM has a higher surface tension in oil droplets from the emulsion step, resulting in larger nanoparticles [20]. Apart from MW, emulsifier concentrations and organic solvent type, power of sonication can affect particle size. The power of sonication applied to the emulsion step can disrupt the surface tension of the larger oil drops to create a smaller oil drop which leads to formation of smaller particles as previously observed [21].

CONCLUSION

Different sizes of PLGA NPs were successfully prepared by changing each of four synthesis parameters. The factors that contribute to the achievement of desirable size are PLGA MW, PVA concentrations, organic solvent type, and sonication power. The NPs obtained are stable in aqueous solution, and therefore are suitable for further development as a drug carrier system for cancer treatment.

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CONFLICT OF INTERESTS

The authors have no conflict of interests.

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