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Note

Uniform-sized PLA nanoparticles: Preparation by premix membrane emulsification

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ABSTRACT

Nanoparticle size is crucial to drug release behavior and biodistribution in vivo, but few studies have been performed on biodegradable nanoparticles with narrow size distribution. In this note, uniform-sized nanoparticles were prepared by a facile method combining emulsion-solvent removal and premix membrane emulsification for the first time. After preparation of coarse emulsions, additional premix membrane emulsification with very high pressure was occupied to achieve uniform-sized nanodroplets, and nanoparticles were formed by further solidification. Polylactide (PLA) was selected as a model polymer. Several factors played key roles to obtain uniform-sized PLA nanoparticles, including type of organic solvent, the volume ratio of oil phase and external water phase, pore size of the microporous membrane and transmembrane pressure. The coefficient of variation (CV) value of PLA nanoparticles could be controlled below 16.9% under an optimum condition. The novel method also has the advantages of high productivity, simplicity and easy scale-up. The uniform-sized nanoparticles prepared by this novel method have great potentials in drug delivery.

Nanoparticles composed of biodegradable polymers are being developed within academic labs as therapeutics (Jin and Ye, 2007; Nishiyama, 2007) and received more and more attentions due to their numerous advantages, including large-specific surface areas, controlled (Leo et al., 2006) and targeted (Panyam and Labhasetwar, 2007) delivery of the drug. The drug can be encapsulated in the particles (Lee et al., 2007), or adsorbed (Jung et al., 2002) or conjugated (Nobs et al., 2003) on the surface.

The particle size is crucial to drug release and biodistribution in vivo (Getierro et al., 2002; Cheng et al., 2007), but limited studies have been performed on biodegradable nanoparticles with narrow size distribution. Typically, the nanoparticles are usually formulated by high-speed homogenization or ultrasonication (Budhian et al., 2007). Indeed, the size of the nanoparticles prepared by these methods is difficult to be controlled and the size distribution is very broad. Consequently, poor reproducibility of the experiments may arise. For instance, although several reports have shown that cellular uptake is correlated with particle size, comparison of the data turned out to be ambiguous and even contradictory due to broad size distribution (Gaumet et al., 2007). Meanwhile, the long-time emulsification generates high energy, which could affect the stability of encapsulated activities (Lim et al., 2003). In order to prepare nanoparticles with narrow size distribution, selective centrifugation was adopted (Gaumet et al., 2007), but the process is tedious and inefficient. Up to now, the preparation method for nanoparticles on an industrial scale has not yet been established. It is still a big challenge to prepare uniform-sized nanoparticles with good reproducibility and easy scale-up.

The objective of this work was to prepare uniform-sized biodegradable nanoparticles for drug delivery with a facile approach. A novel method combined emulsion emulsification and premix membrane emulsification was proposed, the coarse emulsions with big size and broad size distribution were obtained by mild conventional emulsification and then extruded through Shirasu porous glass (SPG) membrane with quite high pressure to form uniform-sized nanodroplets. Finally, nanoparticles were achieved by further solidification. Polylactide (PLA), a type of polymer approved by the Food and Drug Administration for human use, was used as model material.

PLA was purchased from the Institute of Medical Instrument (Shandong, China). Poly(vinyl alcohol) (PVA-217) was ordered from Kuraray (Tokyo, Japan). Shirasu porous glass (SPG) membrane was provided by SPG Technology Co. Ltd. (Sadowara, Japan). All other reagents were of analytical grade.

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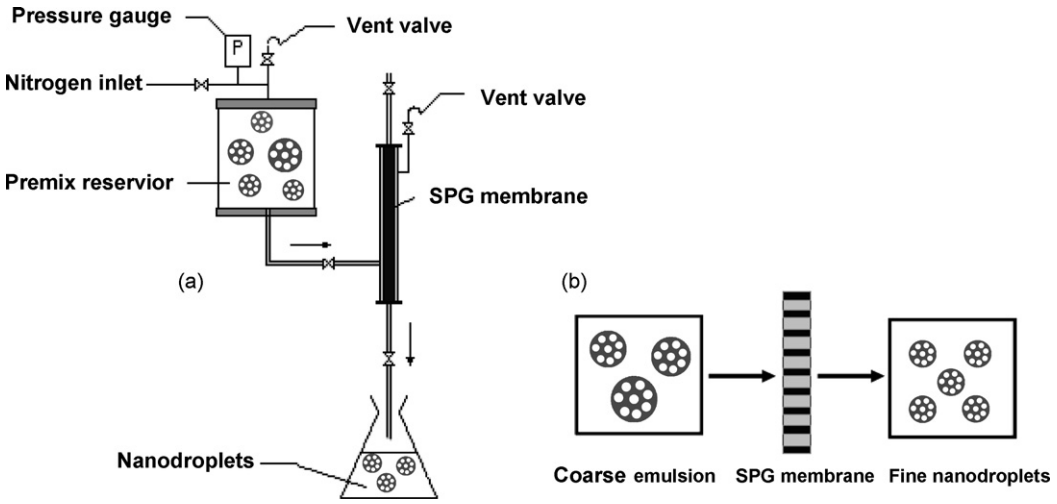

Fig. 1. Schematic presentation of (a) miniature kit and (b) corresponding principle for premix membrane emulsification.

PLA nanoparticles were prepared by two-step procedure. The coarse emulsions were first prepared by low-speed stator homogenization and then poured into the premix reservoir. As schematized in Fig. 1, nanodroplets were achieved by extruding the coarse emulsions through the SPG membrane with quite high pressure. The nanodroplets were stirred overnight to evaporate organic solvent and the obtained PLA nanoparticles were washed three times and collected.

Various factors influencing the size and size distribution of PLA nanoparticles were investigated. Unless specified otherwise, the standard formulation conditions were as following: the pore size of membrane was 1.4 μm, transmembrane pressure was 1000 kPa, and the volume ratio of oil phase and external water phase was 1:5.

The surface morphology of PLA nanoparticles was observed by scanning electron microscope (SEM). The mean size was measured by dynamic light scattering technique, and the particle size distribution was expressed by a coefficient of variation (CV) value, as previously reported (Liu et al., 2005; Wang et al., 2005). The encapsulation efficiency of nanoparticles was determined according to a method previously described (Meng et al., 2003; Liu et al., 2005).

During the process of premix membrane emulsification for preparation of PLA nanoparticles, the size of coarse emulsions was reduced to nanoscale due to high transmembrane pressure for droplets disruption. The obtained nanodroplets would solidify quickly and jam the pore of the microporous membrane when volatile organic solvent was used. Thus, methylene chloride, which is the most often used organic solvent, can not be used for preparation of PLA nanoparticles. Ethyl acetate was selected as organic solvent in this note due to its relatively high boiling point (76.7 °C). In contrast, ethyl acetate is considerably less toxic than methylene chloride and easy to be extracted rapidly with an excess aqueous solution, which favors for bioactivity and encapsulation efficiency of entrapped drug (Meng et al., 2003).

As we know, ethyl acetate shows good solubility in water (8.7%, w/v). Therefore, when external water phase volume increased, a larger amount of ethyl acetate diffused from the oil phase into the external water. Then, the oil droplet became more viscous, which would weaken the extent of droplet disruption of the coarse emulsions. Thus, as shown in Fig. 2, bigger size and broader size distribution were revealed. The mean size of the obtained nanoparticles were about 321 nm, 493 and 669 nm, the CV value were 18.5, 30.0, 43.3%, when the volume ratio of oil phase and external water phase was 1:5, 1:7.5 and 1:10, respectively.

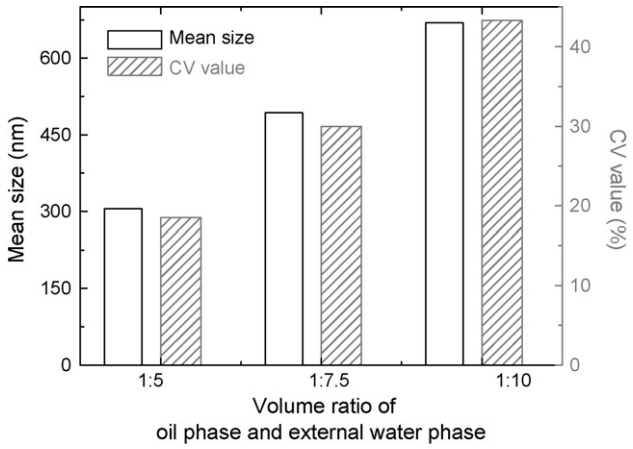

Fig. 2. Effect of volume ratio of oil phase and external water phase.

The pore size of the SPG membrane is also crucial to preparation of uniform-sized nanoparticles. When SPG membrane with a bigger pore size was occupied for premix membrane emulsification, bigger droplets could pass through the SPG membrane easily, which led to bigger size and broader size distribution (Fig. 3). The CV values of them were 18.5, 26.9, 36.3 and 39%, respectively. Herein, small pore size of membrane is required to prepare uniform-sized nanoparticles.

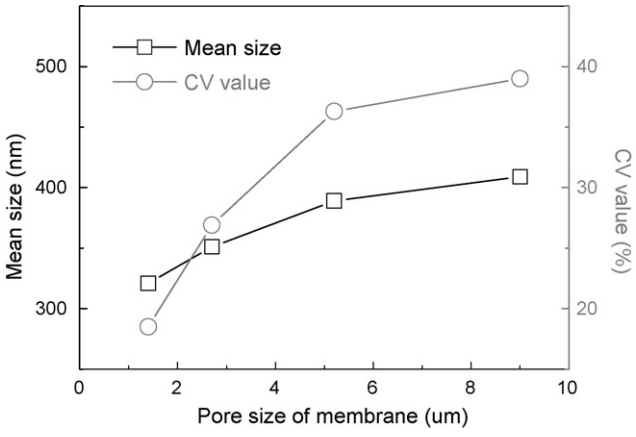

Fig. 3. Effect of pore size of membrane.

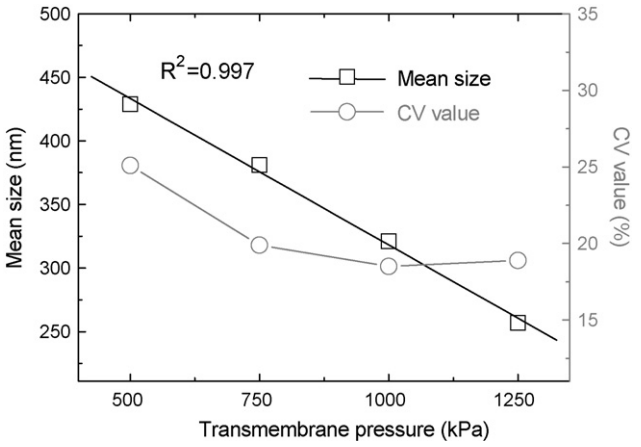

Fig. 4. Effect of transmembrane pressure.

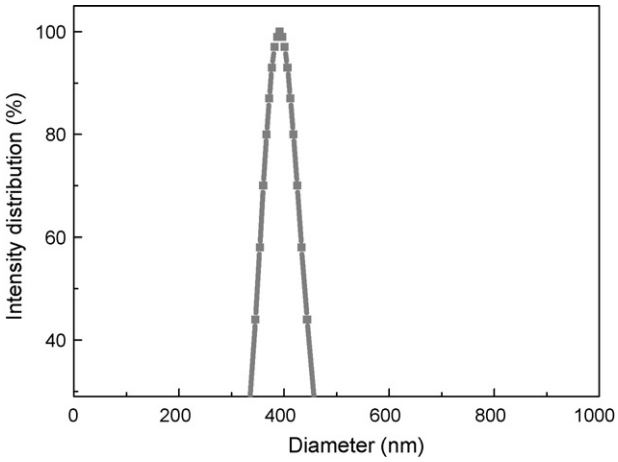

Fig. 6. Size distribution of PLA nanoparticles.

Compared with conventional long-time homogenization and ultrasonication, the premix membrane emulsification with a high pressure led to faster formulation of nanodroplets, which benefited for the stability of encapsulated activities. This higher pressure would lead to harder collisions between coarse emulsion and tortuous pore walls of SPG membrane, which accelerating the break of droplets. As shown in Fig. 4, with the increase of transmembrane pressure, small size and narrow size distribution was obtained. It is obvious that high pressure is absolutely a necessary factor to achieve uniform-sized nanoparticles.

Interestingly, the data between mean size and transmembrane pressure showed a good linear relationship (Fig. 4), which allowed us to formulate uniform-sized nanoparticles with desired sizes to meet different applications.

Scale-up of the method to industrial scale is easy just by adding surface area of the membrane or parallel connecting the membrane in apparatus. The results mentioned above provide an approach to prepare uniform-sized nanoparticles for industrial production.

The results obtained in this study showed that relatively uniform-sized nanoparticles could be prepared by premix membrane emulsification at high pressure. The additional premix membrane emulsification could also be repeated for several times. Under an optimum condition, the CV value of the obtained PLA nanoparticles could be controlled below 16.9% (Figs. 5 and 6). Applying this method to prepare nanoparticles has other advantages besides narrow size distribution and size-controllability, such as its high productivity, simplicity and suitability for any synthetic polymer and natural polymers. The moderate condition and easy scale-up are also appealing to the practical application.

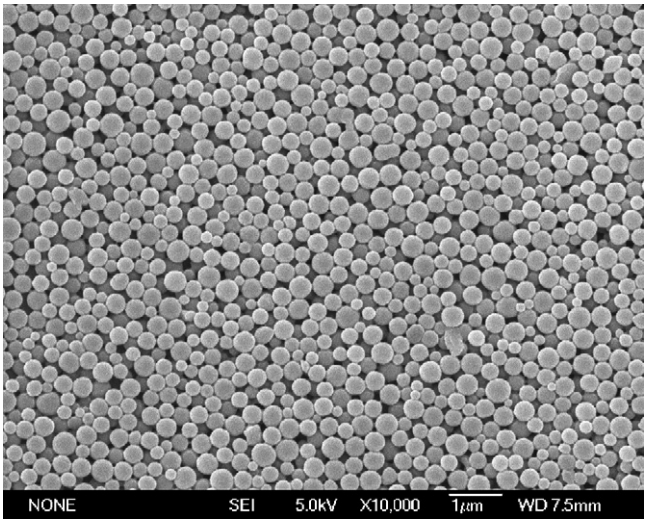

Fig. 5. SEM photographs of PLA nanoparticles.

To validate the feasibility of the novel method for application in drug delivery, Bovine Serum Albumin (BSA) was selected as a model drug and encapsulated into the nanoparticles. The actual loading and encapsulation efficiency of nanoparticles could reach to 8.09 μg BSA/mg particle and 32.3%, respectively, and the uniformity and sphericity of drug-load nanoparticles was not affected evidently, which were satisfied for drug delivery system (Delie et al., 2001; Lecaroz et al., 2006).

Our further study will focus on the application of nanoparticles fabricated by this novel method. Preparation of uniform-sized nanoparticles with other materials, such as chitosan, also will be investigated.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2008.03.027.

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