Polymeric Nano-half-shells prepared by Simple Solvent Evaporation Method

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Nano- and microparticles composed of degradable polymers have been used for drug and gene delivery systems. Recently, polymeric hollow microspheres were developed for pulmonary delivery of drugs and genes. Generally, in an oil-in-water (o/w) emulsion solvent evaporation method has been used for the preparation of poly(D,L-lactide-co-glycolic acid) (PLGA) nano- and microparticles which had spherical shapes, although hollow microspheres can be prepared by other methods such as water-in-oil-in-water (w/o/w) double emulsion solvent evaporation and jet spraying with a nozzle. In this article, we report that nano-half-shells could be prepared by the same oil-in-water emulsion solvent evaporation method by adding Pluronic F127 (poloxamer) to the organic phase. Generally, half-shells are not thought to be produced spontaneously, because of their high surface tension. Thus, poloxamer seems to play a key role in generating the thermodynamically unfavorable structure of nano-half-shells.

PLGA nanoparticles were prepared with an organic phase of methylene chloride containing various amounts of poloxamer (0, 0.5, 1.0, 1.5, 2.0 and 2.5% w/v) respectively with 5% (w/v) PLGA (50:50, Mw 28,032) to verify whether poloxamer could affect the nanostructures. The oil-in-water emulsion was produced by using an ultrasonic processor to emulsify 4 mL of methylene chloride into 40 mL of 1% (w/v) polystyrene. After ultrasonic treatment, the solidified PLGA nanoparticles were washed twice with distilled water by centrifugation at 20,000 rpm for 10 minutes, and lyophilized. Other polymers, such as higher molecular weight PLGA (50:50, Mw 40,000-75,000), PLGA (85:15, Mw 50,000-75,000), polylactide (Mw 85,000-160,000), and polystyrene (Mw 350,000), were dissolved in methylene chloride (3% w/v) respectively with 2.5% (w/v) poloxamer and applied to the same protocol of PLGA nanoparticle preparation.

When PLGA nanoparticles prepared in an organic phase containing PLGA and poloxamer were subject to morphological analysis by scanning electron microscopy, half-shell structures were hardly observed in PLGA nanoparticles with less than 2.5% poloxamer, whereas 2.5% poloxamer-formulated PLGA nanoparticles mostly constructed nano-half-shells which were bigger than other spherical nanoparticles (Figure 1. (A)-(C)). Thus, we think that the poloxamer dissolved in organic phase seems to be important factors in generating such half-shell type PLGA nanoparticles.

To investigate the mechanism of nano-half-shells formation, the amount of poloxamer remained in PLGA nanoparticles was measured by $^1$H NMR spectroscopy. Three major peaks of PLGA and one major peak of poloxamer were found in the $^1$H NMR spectra of PLGA nanoparticles dissolved in CDCl₃. The ratio of integration values was calculated as a parameter for the content of poloxamer in PLGA nanoparticles and is displayed in Figure 2. The poloxamer content increased with the amount of poloxamer used in the preparation solution in the cases of poloxamer-formulated PLGA nanoparticles. This result suggested that nano-half-shells were not generated by the removal of poloxamer during preparation, since the nano-half-shells exhibited a high content of

![Figure 1](image-url) Morphological analysis of PLGA nanoparticles by scanning electron microscopy. (A) 0%, (B) 0.5%, (C) 2.5% poloxamer-formulated respectively. The bars indicate 1 μm.

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poloxamer. On the contrary, the structures of nano-half-shells were thought to be closely related with the high incorporation efficiency of poloxamer, which was compared by calculating the integral values ratios of poloxamer-formulated PLGA nanoparticles and those of comparative groups (a) higher molecular weight PLGA, (b) PLGA (85:15), (c) polylactide, and (d) polystyrene, (e) 0% poloxamer-formulated PLGA nanoparticles prepared using the outer water phase containing 2.5% (w/v) poloxamer, (f) 2.5% poloxamer-formulated PLGA nanoparticles prepared using the outer water phase containing 2.5% (w/v) poloxamer.

Figure 2. Integration ratios of the poloxamer peak and the PLGA peak in 1H NMR spectra, (■) the standard solutions (CDCl3) dissolving poloxamer and PLGA as preparation solutions, (○) the range of 0% to 5.0% poloxamer-formulated PLGA nanoparticles.

Figure 3. Incorporation efficiencies of poloxamer in the range of 0.5% to 5.0% poloxamer-formulated PLGA nanoparticles and those of comparative groups, (a) higher molecular weight PLGA, (b) PLGA (85:15), (c) polylactide, and (d) polystyrene, (e) 0% poloxamer-formulated PLGA nanoparticles prepared using the outer water phase containing 2.5% (w/v) poloxamer, (f) 2.5% poloxamer-formulated PLGA nanoparticles prepared using the outer water phase containing 2.5% (w/v) poloxamer.

Thus, one of the unique properties of the organic phase containing PLGA and poloxamer could be its water-trapping ability. Poloxamer is thought to be diffused to the outer water phase during emulsification at a slower rate after trapping water into the organic phase, which it does more efficiently than polyethylene glycol because of its amphiphilic nature. Moreover, the hydrophilic property of PLGA might enhance this water-trapping in the organic phase, which seems to cause fast solidification at the surface and the creation of the hollow structure. This suggestion of fast solidification could also explain the higher incorporation ratio of poloxamer and the bigger size in nano-half-shells. Thus, nano-half-shells were thought to be generated during the solidification stage.

The suggested mechanism for nano-half-shell formation is as follows, and is depicted in Scheme 1. Initially, water is diffused into the organic phase and methylene chloride evaporates as soon as the emulsion is created (A). At the same time, the surface of the emulsion solidifies quickly depending on the water entrapment ability of the organic phase (B), which corresponds to a rapid solvent removal phase in the literature. In this stage, since methylene chloride can be evaporated and water can be diffused into the inner phase through the thin solidified film, the empty space left by methylene chloride evaporation is replaced by water until the point at which the two phases are separated and an inner water emulsion is created (C). This structure is basically the same as the structure of the
water-in-oil-in-water double emulsion method. At this stage, similar to a solvent removal phase, a viscous layer of polymer and water emulsion can be stabilized by the surfactant effect of poloxamer, which seems to be allocated at a defined ratio between water and methylene chloride. This suggestion also explains why all nano-half-shells show similar incorporation efficiencies over 50%. Then, if this inner water is in contact with any part of the emulsion surface, water may move to the outer aqueous phase forming a hole of which the size depends on the amounts of the entrapped water (D). Then, the inner surface of the organic polymer layer would also solidify, and the remaining methylene chloride would evaporate through both the outer and inner surface (E), and finally nano-half-shells are generated (F).

Since these processes, after water uptake, corresponded basically to the suggestions in the literature,14 the poloxamer was considered to produce the porous hollow microspheres if a mild emulsifying method such as stirring was used instead of an ultrasonic processor. Actually, the porous hollow microspheres could be prepared by adding 2.5% (w/v) poloxamer to the organic phase of 5% (w/v) PLGA with stirring (data not shown).

In conclusion, various PLGA nanoparticles including nano-half-shells were prepared by the oil-in-water emulsion solvent evaporation method and the mechanism of nano-half-shell formation is suggested to be a series of events including fast solidification, phase separation, and water escape. Since these nano-half-shells are nanostructures with low densities, they have the possibility of being used as carriers for pulmonary drug delivery and so trials along these lines of inquiry are now in progress.

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