Controlling Particle Size of Poly(lactic acid)/Hydroxyapatite Nanoparticles

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Nanoparticles have gained immense attention as drug carriers in biodegradable drug delivery systems. Particle size is one of the most important characteristics of nanoparticles. It determines the *in vivo* distribution and targeting ability of nanoparticle drug delivery systems [1,2]. Over the past few years, biodegradable polymers have gained significant attention for developing biodegradable nanoparticle drug delivery systems. Poly(lactic acid) (PLA) is one of the most promising biodegradable polymers and has been used as a carrier in drug delivery applications as it decomposes safely in the body [3]. Various polymers such as poly(lactic acid) and poly(lactic acid-co-glycolic acid) have been used for drug delivery applications as they can effectively deliver drugs to the target sites [4,5]. These particles are usually prepared using the solvent evaporation method. In this method, an oil-in-water emulsion containing an organic phase capable of dissolving various polymers and a water phase dissolving the surface-active agent is used. After the formation of the emulsion, the organic solvent evaporates, resulting in the formation of the polymer particle. However, in this method, the non-biodegradable surface-active agents remain in the resulting polymer particles [5]. Nagata et al. developed a surfactant-free method for the preparation of poly(lactic acid)/hydroxyapatite core-shell particles for drug delivery applications [6–8]. Hydroxyapatite (HAp; Ca_{10}(PO_{4})_{6}(OH)_{2}) is a calcium phosphate and is the major mineral component of human bones and teeth. Hence, owing to its biocompatibility and absorbability with many compounds [9–11], HAp has gained immense attention for applications such as biomaterials, ion exchangers, adsorbents, and catalysts [12–14]. Moreover, PLA/HAp particles decompose safely in the body because of their surfactant-free preparation. Thus, the PLA/HAp particles prepared by the surfactant-free method are potential drug delivery carriers. However, it is important to control their particle size for drug delivery applications. In this study, we investigated the effect of the PLA content and ionic concentrations on the particle size of PLA/HAp particles. Here, PLA/HAp particles with different PLA contents and ionic concentrations was determined by SEM. The results showed that the PLA content played a crucial role in increasing the particle size of the PLA/HAp particles.

Key words: Poly(lactic acid), Hydroxyapatite, Particle Size, Surfactant-Free

1. INTRODUCTION
Nanoparticles have gained immense attention as drug carriers in biodegradable drug delivery systems. Particle size is one of the most important characteristics of nanoparticles. It determines the *in vivo* distribution and targeting ability of nanoparticle drug delivery systems [1,2].

Over the past few years, biodegradable polymers have gained significant attention for developing biodegradable nanoparticle drug delivery systems. Poly(lactic acid) (PLA) is one of the most promising biodegradable polymers and has been used as a carrier in drug delivery applications as it decomposes safely in the body [3]. Various polymers such as poly(lactic acid) and poly(lactic acid-co-glycolic acid) have been used for drug delivery applications as they can effectively deliver drugs to the target sites [4,5]. These particles are usually prepared using the solvent evaporation method. In this method, an oil-in-water emulsion containing an organic phase capable of dissolving various polymers and a water phase dissolving the surface-active agent is used. After the formation of the emulsion, the organic solvent evaporates, resulting in the formation of the polymer particle. However, in this method, the non-biodegradable surface-active agents remain in the resulting polymer particles [5].

Nagata et al. developed a surfactant-free method for the preparation of poly(lactic acid)/hydroxyapatite core-shell particles for drug delivery applications [6–8]. Hydroxyapatite (HAp; Ca_{10}(PO_{4})_{6}(OH)_{2}) is a calcium phosphate and is the major mineral component of human bones and teeth. Hence, owing to its biocompatibility and absorbability with many compounds [9–11], HAp has gained immense attention for applications such as biomaterials, ion exchangers, adsorbents, and catalysts [12–14]. Moreover, PLA/HAp particles decompose safely in the body because of their surfactant-free preparation. Thus, the PLA/HAp particles prepared by the surfactant-free method are potential drug delivery carriers. However, it is important to control their particle size for drug delivery applications. In this study, we investigated the effect of the PLA content and ionic concentrations on the particle size of PLA/HAp particles.

2. MATERIALS AND METHODS
2.1 Materials
Calcium acetate monohydrate (Ca(CH_{2}COO)_{2}·H_{2}O), diammonium hydrogen phosphate ((NH_{4})_{2}HPO_{4}), PLA-0020 (molecular weight = 20,000), and acetone were purchased from Wako Pure Chemical Industries, Ltd., Japan. All reagents were used as-purchased without further purification.

2.2 Preparation of PLA/HAp particles
PLA/HAp particles were prepared by the surfactant-free emulsification method. PLA was dissolved in acetone. PLA solutions with various PLA contents (20, 40, 80, 250, 500 mg) were prepared. These solutions were added in distilled water. Ca(CH_{2}COO)_{2}·H_{2}O was added to the resulting mixtures in various concentrations (20–80 mM). To the resulting mixtures, (NH_{4})_{2}HPO_{4} was added (12–48 mM) with magnetic stirring to set the Ca/P molar ratio to 1.67. These mixtures were then kept at room temperature
for 24–72 h in order to remove the solvent. The samples were collected by filtration and then washed with distilled water. The synthesis conditions are listed in Table I.

Table I. Synthesis conditions for the PLA/HAp particles.

<table>
<thead>
<tr>
<th>synthetic conditions</th>
<th>PLA (mg)</th>
<th>Ca(CH₃COO)₂ (mM)</th>
<th>(NH₄)₂HPO₄ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLHA-20</td>
<td>20</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>PLHA-40</td>
<td>40</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>PLHA-80</td>
<td>80</td>
<td>80</td>
<td>48</td>
</tr>
<tr>
<td>PLHA-250-20</td>
<td>250</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>PLHA-250-40</td>
<td>250</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>PLHA-500-20</td>
<td>500</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>PLHA-500-40</td>
<td>500</td>
<td>40</td>
<td>24</td>
</tr>
</tbody>
</table>

2.3 Characterization of PLA/HAp particles

Morphology and particle size: The morphology and particle size of all the samples were analyzed by field emission scanning electron microscopy (FE-SEM, Hitachi, Ltd., S4300, Japan) at an accelerating voltage of 10 kV. The samples were placed on the sample holder and were sputter-coated with platinum in order to minimize the sample-charging problems. SEM images were used to measure the diameter of the PLA/HAp core-shell particles (in dried state) (n=100). The resulting data are presented as mean size ± standard deviations.

Analysis of the chemical composition of PLA/HAp particles: The chemical composition of the particles was analyzed by Fourier transform infrared spectroscopy (FTIR 4700 JEOL, Ltd., Japan) using the attenuated total reflection unit at a resolution of 4 cm⁻¹ at room temperature.

Observation of the emulsion size: Emulsion sizes were determined using a dynamic light scattering (DLS) detector (ELS-1000) (Otsuka Electronics Co., Ltd.) at room temperature. PLA solutions with various concentrations were prepared. The solutions were added to distilled water. The resulting mixtures were kept at room temperature for 10 min before measuring their emulsion sizes by DLS. The viscosity and refraction indices of the emulsions were set equal to those of water.

HAp:PLA ratio of PLA/HAp composites: The HAp:PLA ratio in the PLA/HAp composites was determined by thermogravimetric analysis (Thermo Plus TG 8120, Rigaku Co., Japan). Heating was performed in air using a platinum holder. The composites were heated up to 1000 °C. The following heating rates were used: 10 °C/min from room temperature to 400 °C, 1 °C/min from 400 to 600 °C, and 10 °C/min from 600 to 1000 °C. PLA decomposed at temperatures above 1000 °C. Thus, the HAp:PLA concentration ratio of the composites was determined.

3. RESULTS AND DISCUSSION

3.1 Chemical composition of PLA/HAp composites

The chemical bonding of the samples was analyzed by FT-IR spectroscopy. The FT-IR spectrum of pure PLA (Fig. 1) showed an adsorption peak at 1751 cm⁻¹ corresponding to its carbonyl groups. In the case of HAp, the adsorption bands corresponding to PO₄³⁻ (O-P-O bending) were observed at 561 and 601 cm⁻¹. The FT-IR spectra confirmed the presence of both PLA (organic) and HAp (inorganic) in PLHA-20. This shows that the PLA/HAp composites were successfully obtained. The bands corresponding to PLA and HAp were observed in all the samples (Fig. 2).

Fig. 1. FT-IR spectra of pure HAp, pure PLA, and PLA/HAp composite.

Fig. 2. FT-IR spectra of the PLA/HAp composites: PLHA-20, PLHA-40, and PLHA-80.

3.2 Morphology and particle size of PLA/HAp composites

We investigated the effect of the PLA content and ionic concentration on the particle size of the PLA/HAp composites. Figure 3 shows the SEM images of the PLA/HAp composites with various PLA contents and ionic concentrations. Figure 3(a) shows the SEM image of PLHA-20, which was prepared using 20 mg PLA, 20 mM Ca(CH₃COO)₂, and 12 mM (NH₄)₂HPO₄. The
sample had spherical particles (Fig. 3 (a)) with an average diameter of $33 \pm 22$ nm. Figure 3 (b) shows the SEM image of PLHA-40 (prepared using 40 mg PLA, 40 mM Ca(CH$_3$COO)$_2$, and 24 mM (NH$_4$)$_2$HPO$_4$). This sample also showed spherical particles (Fig. 3 (b)). The average diameter of these particles was $34 \pm 24$ nm. In contrast, PLHA-80 (prepared using 80 mg PLA, 80 mM Ca(CH$_3$COO)$_2$, and 48 mM (NH$_4$)$_2$HPO$_4$) showed no spherical particles (Fig. 3 (c)). The SEM image of this sample showed some flaky products. These products were formed by homogeneous precipitation in the solutions because of their high ionic concentrations. This indicates that high ionic concentrations resulted in the formation of unexpected flaky precipitates. Therefore, we used 20 and 40 mM Ca(CH$_3$COO)$_2$ and 12 and 24 mM (NH$_4$)$_2$HPO$_4$ aqueous solutions. We also investigated the effect of the amount of PLA on the particle size of the composites. The SEM images shown in Figs. 4 (a) and (b) show that the PLHA-250-20 and PLHA-250-40 samples had spherical particles with an average particle size of $74 \pm 37$ and $77 \pm 56$ nm, respectively. This indicates that an increase in the amount of PLA added to the composite increased its particle size. The PLHA-500-20 and PLHA-500-40 samples showed no spherical particles (Figs. 4 (c) and (e)). The SEM images of these samples showed that the PLA particles in these samples fused together. This is because the excess PLA in these samples resulted in the formation of an incomplete HAp shell.

### 3.3 Emulsion size

The SEM images of the PLA/HAp samples show the effect of the PLA content on their particle size (Figs. 3 and 4). The PLA concentration in the acetone solution affected the size of the resulting PLA/HAp particles. The effect of the emulsion particles on the PLA concentration was also investigated. PLA solutions with various concentrations were prepared. The solutions were added to distilled water. The resulting mixtures were kept at room temperature for 10 min, before measuring their emulsion sizes by DLS. Figure 5 shows that an increase in the PLA concentration from 5 to 20 mg/mL increased the average diameter of its emulsion particles from 83 to 164 nm. (These polydispersity indexes were 0.102 and 0.076, respectively.) This shows that the emulsion size of PLA was affected by its concentration in the acetone solution. Hence, the particle size of the PLA/HAp particles could be increased by increasing the PLA concentration.

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**Fig. 3.** SEM images of the PLA/HAp composites with different amounts of PLA, (a) PLHA-20, (b) PLHA-40, and (c) PLHA-80.

**Fig. 4.** SEM images of PLA/HAp composites prepared with different amount of PLA and ionic concentrations. (a, b) PLHA-250, (c–e) PLHA-500.

**Fig. 5.** Relationship between average diameter of the PLA emulsion particle and the concentrations of PLA in acetone solutions.
3.4 HAp:PLA ratio of PLA/HAp composites
The HAp:PLA ratio of the PLA/HAp composites was determined by TG analysis. Figure 6 shows the TG profiles of the HAp/PLA composites (PLHA-20, -40 and -80). The composites showed a sharp weight loss over the temperature range of 200–300 °C. This weight loss is attributed to the thermal decomposition of PLA. Therefore, the HAp:PLA ratio could be determined by estimating the residual weights of these samples. The HAp:PLA ratio of the PLA/HAp PLHA-20, PLHA-40, and PLHA-80 composites was calculated to be about 60:40, 50:50, and 47:53 respectively. This result indicates that an increase in ionic concentrations added to the calcium phosphate increased its particle.

![Figure 6. TG curves of the PLA/HAp composites: (a) PLHA-20, (b) PLHA-40, and (c) PLHA-80.](image)

4. CONCLUSION
In this study, we investigated the effect of PLA content and ionic concentrations on the particle size of PLA/HAp particles. The FT-IR results showed the presence of both PLA and HAp in all the samples. The PLA/HAp composites were successfully obtained. The average diameters of PLHA-20 and PLHA-250-40, as calculated from their SEM images, were 34 ± 24 nm and 77 ± 56 nm, respectively. The average diameter of the composites was affected by their PLA contents. It was found that the size of the PLA emulsions increased with an increase in their PLA concentrations. In addition, the size of the PLA/HAp particles was affected by their PLA contents.

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5. REFERENCES

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