Effect of the Conformation of Poly(L-lactide-co-glycolide) Molecules in Organic Solvents on Nanoparticle Size

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Abstract: Controlling the size of nanoparticles is important for drug delivery methods such as pulmonary administration, transdermal administration, and intravenous administration. In this study, we have investigated the effect of polymer conformation in organic solvents on the size of the nanoparticles. Poly(L-lactide-co-glycolide) (PLLGA), a promising nanoparticle carrier, was used as the polymer. A mixed solution of dichloromethane, which is a good solvent, and a lower alcohol (methanol, ethanol, and 1-propanol), which is a poor solvent, was used as the solvent for dissolving PLLGA. An oil-in-water emulsion was prepared by sonication using the mixed solution of organic solvents in which PLLGA was dissolved as a dispersed phase and an amino acid aqueous solution as a continuous phase. Nanocomposite particles were prepared from the emulsion using a spray dryer and redispersed in purified water to obtain the PLLGA nanoparticles. The conformation of PLLGA molecules in the organic solvents was evaluated by analyzing the results of the viscosity measurements. The polymer coil radius and the volume per polymer coil were observed to decrease with the increase in the ratio of the lower alcohol in the solvent, whereas these values tended to decrease with the use of more hydrophilic lower alcohols. In addition, based on the results of the calculated entanglement index, it was found that when the hydrophobicity of the dispersed phase is reduced, the polymers were hardly entangled with each other. These results were significant, specifically when the ratio of the lower alcohol in the solvent was low. Estimation of the Pearson’s correlation coefficients indicated that there were positive correlations between these indices and the mean volume diameter of PLLGA nanoparticles. This study shows that changing the composition of the dispersed phase, in which the PLLGA is dissolved, can change the conformation of the PLLGA molecules and control the size of the PLLGA nanoparticles.

Key words: poly(L-lactide-co-glycolide), nanoparticle, organic solvent, intrinsic viscosity, polymer coil

1 Introduction

Fine particles play an important role in drug delivery systems. It is widely known that parenteral drug administration is expected to improve bioavailability by avoiding hepatic first-pass effects that can prematurely metabolize drugs¹. The lungs have a promising drug delivery route due to their large surface area and thin absorption barrier². Pulmonary administration of a drug can be expected to have local and systemic effects, and nano- to micro-sized particles are generally used as drug carriers³–⁴. Desirable product characteristics of the particles involved in pulmonary administration include correct aerosolization properties, such as low mass average aerodynamic diameter, high fine particulate fraction, and high emitted dose. To control these, fine particle preparation using polymers has been studied⁵,⁶. The skin is also a promising route for systemic and local delivery of therapeutic agents⁷. The stratum corneum, the outermost layer of the epidermis, is a critical barrier that limits the delivery of many drugs; therefore, transdermal drug delivery systems using various types of nano-sized carriers have been reported to deliver sufficient amounts of drugs into the deeper layers⁸,⁹. To deliver drugs deep into the skin while maintaining normal skin barrier function, the use of conventional chemical penetration enhancers and physical enhancement techniques should be avoided as much as possible. As a result, polymer

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nanoparticle delivery systems using polymers as drug carriers are attracting attention\(^9\). Such systems have been used in the studies of boron neutron capture therapy (BNCT). In a biodistribution study on tumor-bearing mice, it was reported that the size of the nanoparticles used as boron carriers affected the biodistribution of boron\(^10\). Fine particles using a polymer are useful as a drug carrier and it is desired to control the particle size since it greatly affects their drug delivery ability. In addition, surface modification is effective for improving the efficacy of nanoparticle preparation. However, this causes a change in particle size\(^11, 12\). Therefore, to make the particle size uniform in a comparative experiment of various particles, it is necessary to carefully adjust the particle size when preparing the core nanoparticle.

Poly(lactide-co-glycolide) (Fig. 1a), which is a class of biodegradable polymers, has attracted considerable attention as a drug carrier because of its biocompatibility and biodegradability\(^13, 14\). In a previous study, we revealed the effect of poly(D,L-lactide-co-glycolide) (PLGA) molecule conformation in the feed solution on the aerodynamic diameter of PLGA microparticles prepared using a spray-drying method. A mixture of dichloromethane, which is a good solvent for PLGA, and methanol, which is a poor solvent for PLGA, was used as a feed solution at an arbitrary ratio. It was shown that as the ratio of the poor solvent in the feed solution increased, the conformation of the polymer in the feed solution became more compact and the size of the prepared microparticles became smaller\(^15\). In this study, we aim to further control the size of the nanoparticles by using three kinds of lower alcohols, namely, methanol, ethanol, and 1-propanol, as poor solvents. We have prepared the nanoparticles using nanocomposite particle preparation technique for inhalation\(^3\). This method is based on the study of inhalable nanocomposite particles reported by Tomoda et al.\(^16\). Typically, nanoparticles are not suitable for inhalation due to their small size. They can gain aerodynamic diameters suitable for inhalation when they are in the form of nanocomposite particles\(^3\). After reaching the alveoli, the nanoparticles are released from the nanocomposite particles by the dissolution of the diluent in the alveolar lining fluid\(^17\). Inhalable nanocomposite particles composed of drug-loaded PLLGA nanoparticles and amino acids (arginine and leucine) were prepared using spray drying via an oil-in-water (O/W) emulsion. A schematic diagram of inhalable nanocomposite particles via an O/W emulsion is shown in Fig. 1b. In this method, since the nanocomposite particles are prepared directly from the O/W emulsion, it is assumed that the contained nanoparticles are easily affected by the dispersed as well as the continuous phase of the emulsion\(^3\). Moreover, in the previous study, it was difficult to compare particle sizes because some of the microparticles were non-spherical, whereas the compared nanoparticles prepared via

![Fig. 1](image)

**Fig. 1** a) Poly(L-lactide-co-glycolide), b) Schematic diagram of O/W emulsion and inhalable nanocomposite particles.

nanocomposite particles were spheroidized. As a polymer, poly(D,L-lactide-co-glycolide) (PLLGA), which is useful for suppressing drug release from the nanoparticles, was used\(^18\). Rifampicin (RFP) was used as a hydrophobic model drug.

### 2 Experimental Procedure

#### 2.1 Materials

PLLGA with a molecular weight of 10,000 and an L-lactic acid/glycolic acid monomer composition of 75/25 was purchased from Taki Chemical Co., Ltd. (Kakogawa, Japan). RFP (C<sub>43</sub>H<sub>58</sub>N<sub>4</sub>O<sub>12</sub>, purity ≥ 97%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Dichloromethane (DCM, purity ≥ 99.5%), methanol (MeOH, purity ≥ 99.8%), ethanol (EtOH, purity ≥ 99.5%), 1-propanol (PrOH, purity ≥ 99.5%), L(+)-arginine (purity ≥ 98%), and L-leucine (purity ≥ 99%) were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). All other commercially available chemicals were of the highest grade.

#### 2.2 Preparation of the PLLGA nanoparticles

The preparation of the nanocomposite particles to obtain the PLLGA nanoparticles was performed in a manner similar to our previous work\(^3\). A dispersed phase was prepared by dissolving 100 mg RFP and 400 mg PLLGA in 10 mL of organic solvents. As the organic solvent, DCM, a mixed solution of DCM and MeOH, DCM and EtOH, or DCM and PrOH was used. The solution was added to 50 mL of aqueous solution in which 500 mg of amino acids (arginine: leucine = 1: 6) was dissolved. The mixed solution
was emulsified in an ice bath using a probe sonicator (Digital Sonifier S-250D, Branson Ultrasonics Corp., Danbury, CT, USA) for 20 s at 200 W of energy output. The O/W emulsion was then spray-dried using a spray dryer (Mini Spray Dryer B-290, BÜCHI Corp., Flawil, Switzerland) to prepare the nanocomposite particles at the outlet temperature of 37–40°C, air volume of 22.5 m³/h and solution sending speed of 1.38 mL/min. The organic solvent in which amino acids were dissolved were quickly removed by spray drying, and the particles (inhalable nanocomposite particle) in which the drug-containing PLLGA nanoparticles and the diluent surrounding them were mixed were obtained. After freezing at -30°C, nanocomposite particles were lyophilized using a freeze dryer (FD-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for 12 h. The mean volume diameters and the size distributions of the PLLGA nanoparticles redispersed from the nanocomposite particles were observed using a scanning electron microscope (SEM, JSM-6060LA, JEOL Ltd., Akishima, Japan).

2.3 Viscosity measurement of the dispersed phase

To evaluate the conformation of the PLLGA molecules in the dispersed phase, a viscometer (TV-20, Toki Sangyo Co., Ltd., Tokyo, Japan) was employed to carry out the viscosity measurements. Since viscosity is affected by temperature, all measurements were performed at 25°C. The intrinsic viscosity ([η]) of PLLGA in the various solvent compositions was obtained using the following expression:

\[ [\eta] = \lim_{c \to 0} \left( \frac{\eta_v}{c} \right) \]

where \( \eta_v \) is the specific viscosity and \( c \) is the concentration of the polymer solution. The polymer coil radius (\( R_{\text{coil}} \)) and the volume per polymer coil (\( V_{\text{coil}} \)) in the various solvents were calculated using the following expressions:

\[ R_{\text{coil}} = \left[ \frac{3[\eta] \cdot M_\text{p}}{10\pi N_\text{A} \cdot c} \right]^{1/3} \]

\[ V_{\text{coil}} = \left( \frac{4}{3} \right) \pi (R_{\text{coil}})^3 \]

where \( M_\text{p} \) is the molecular weight of the polymer and \( N_\text{A} \) is the Avogadro’s number.

An entanglement index, which is an index used for evaluating the degree of entanglement between polymers in a solution, was also obtained using [\( [\eta] \)]. The overlap concentration (\( c^* \)) in the various solvents was calculated as the reciprocal of [\( [\eta] \)]. Therefore, the entanglement index can be expressed as

\[ \text{Entanglement index} = \frac{C_{\text{polymer}}}{c^*} \]

where \( C_{\text{polymer}} \) is the concentration of PLLGA in the dispersed phase.

3 Results and Discussion

3.1 Characterization of the PLLGA nanoparticles

Various PLLGA nanoparticles were successfully prepared using the technique described in Section 2.2. Their mean volume diameter, polydispersity index, and coefficient of variation are summarized in Table 1. From the obtained values of the polydispersity index and the coefficient of variation, the effect of the composition of the dispersed phase was not clear. Further, the size of the nanoparticles was observed to decrease with the increasing ratio of lower alcohol in the solvent. Increasing the ratio of lower alcohol in DCM reduced the size of the prepared nanoparticles to approximately 60% of the size of nanoparticles prepared using DCM alone. The RFP content in the nanoparticles prepared using only DCM as the dispersed phase was 4.8 ±
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**Fig. 2** Particle size distribution of PLLGA nanoparticles when dichloromethane (DCM) and lower alcohol are used for the dispersed phase (n = 3). (a) Effect of changing the ratio of methanol (MeOH) in the dispersed phase on particle size. (b) Effect of changing the ratio of ethanol (EtOH) in the dispersed phase on particle size. (c) Effect of changing the ratio of 1-propanol (PrOH) in the dispersed phase on particle size.

**Fig. 3** Scanning electron microscopy images of PLLGA nanoparticles taken at an accelerating voltage of 15 kV (magnification: 20,000 ×). (a) Bare nanoparticles.

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0.1% (entrapment efficiency: 24.0 ± 0.5%). When the ratio of MeOH, EtOH, and PrOH in the dispersed phase was increased to 30%, the RFP content decreased to 4.8 ± 0.2, 4.6 ± 1.4, and 3.9 ± 0.5% respectively. This indicates that the transferability of RFP to the amino acid aqueous solution, which is a continuous phase, was improved due to the increased hydrophilicity of the dispersed phase. However, since the RFP content decreased as the number of carbon atoms in the alcohol increased, it is necessary to consider the conformation of PLLGA in the dispersed phase. The particle size distributions of PLLGA nanoparticles are shown in Fig. 2. From the results of Figs. 2(a)–2(c), it was found that as the ratio of the lower alcohol in the dispersed phase increased, the particle size distribution shifted to the left and the degree of shift tended to be larger for lower alcohols having a larger number of carbon atoms. Figure 3 shows a few representative SEM images. From these results, we have confirmed that PLLGA nanoparticles were spherical dispersed particles. L-Leucine was added to reduce the aggregability of the nanocomposite particles as the polymers formed in the solvent. In solvents having a ratio of DCM to lower alcohol of 9:1 and 7:3, these values tended to decrease with lower hydrophobicity of the lower alcohol. R coil was found that as the ratio of the lower alcohol in the dispersed phase, the polymer shrinks in the dispersed phase, the nanoparticles prepared become smaller. In addition, it is considered that the increased hydrophilicity of the organic phase improves the dispersion efficiency of the hydrophobic polymer in the aqueous medium, which also contributes to the formation of smaller nanoparticles.

3.2 Effects of the conformation of PLLGA molecules in organic solvents on the particle size

Figure 4 shows the relationship between the ratio of lower alcohols in the organic solvents as the dispersed phase and the mean volume diameter of the nanoparticles. The correlativity was calculated using a general method of linear regression analysis. R2 values, which are the determination coefficients, of a mixed solution of DCM and MeOH, DCM and ErOH, and DCM and PrOH were 0.939, 0.994, and 0.975 respectively. It was suggested that there was a correlation between the ratio of the lower alcohol in the dispersed phase and the mean volume diameter of the PLLGA nanoparticles. Table 2 shows the properties of the PLLGA molecules in organic solvents used as a dispersed phase for nanoparticle preparation. R coil and V coil were seen to decrease with the increase in the ratio of lower alcohol, which is a poor solvent for PLLGA. In solvents having a ratio of DCM to lower alcohol of 9:1 and 7:3, these values tended to decrease with lower hydrophobicity of the lower alcohol. R coil and V coil were used to estimate the conformation of the polymers formed in the solvent. These results support the previous study on microparticles. It has been reported that a polymer expands in a good solvent and contracts in a poor solvent. Table 2 also shows that the higher the ratio of the lower alcohol in the dispersed phase, the higher in the value of c* and the lower is the entanglement index. This suggests that it becomes difficult for the polymers to be entangled with each other as the hydrophobicity of the dispersed phase decreases. Figure 5 shows the relationship of the mean volume diameter with R coil, V coil and the entanglement index. The Pearson’s correlation coefficients (r) were 0.71, 0.69, and 0.68 for the mean volume diameter and R coil, the mean volume diameter and V coil, and the mean volume diameter and the entanglement index, respectively. Thus, it was found that all the parameters had a positive correlation. From these results, it was suggested that as the polymer shrinks in the dispersed phase, the nanoparticles prepared become smaller. In addition, it is considered that the increased hydrophilicity of the organic phase improves the dispersion efficiency of the hydrophobic polymer in the aqueous medium, which also contributes to the formation of smaller nanoparticles.

4 Conclusion

In this study, we demonstrated that changing the composition of the dispersed phase using lower alcohols can control the size of the PLLGA nanoparticles. The results of the viscosity measurement showed that in the preparation of the PLLGA nanoparticles, a decrease in the ratio of good solvent reduced the spread of the polymer. This suggests that when preparing nanoparticles using hydrophobic polymers, it is necessary to select organic solvents with the
optimal hydrophobicity in order to achieve the desired particle size. In the dispersed phase having a high ratio of lower alcohols, it is necessary to consider their interaction with the continuous aqueous phase and hence further studies need to be carried out.

![Table 2](image)

### Table 2: Properties of PLLGA molecules in organic solvent used as dispersed phase for nanoparticle preparation.

|  | \(\eta\) (dL/g) | \(R_{\text{coil}}\) (nm) | \(V_{\text{coil}}\) (nm\(^3\)) | \(c^*\) (g/dL) | Entanglement index |
|---|---|---|---|---|---|
| DCM only | 0.32 | 1.30 | 9.18 | 3.13 | 1.28 |
| DCM: MeOH = 9: 1 | 0.23 | 1.17 | 6.65 | 4.35 | 0.92 |
| DCM: MeOH = 7: 3 | 0.21 | 1.14 | 6.12 | 4.76 | 0.84 |
| DCM: EtOH = 9: 1 | 0.23 | 1.17 | 6.67 | 4.35 | 0.92 |
| DCM: EtOH = 7: 3 | 0.21 | 1.14 | 6.19 | 4.76 | 0.84 |
| DCM: PrOH = 9: 1 | 0.29 | 1.26 | 8.32 | 3.45 | 1.16 |
| DCM: PrOH = 7: 3 | 0.23 | 1.17 | 6.71 | 4.35 | 0.92 |
| DCM: PrOH = 5: 5 | 0.21 | 1.13 | 6.09 | 4.76 | 0.84 |

![Fig. 5](image)

**Fig. 5** Relationship between the mean volume diameter of PLLGA nanoparticles (x-axis) and the polymer coil radius \(R_{\text{coil}}\) (a), the volume per polymer coil \(V_{\text{coil}}\) (b), or entanglement index \(c^*\) (c) in the various solvents (y-axis). Solid lines indicate regression lines.
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Conflict of Interest
The authors declare that they have no potential conflicts of interest.

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