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# Effects of the conformation of PLGA molecules in the organic solvent on the aerodynamic diameter of spray dried microparticles

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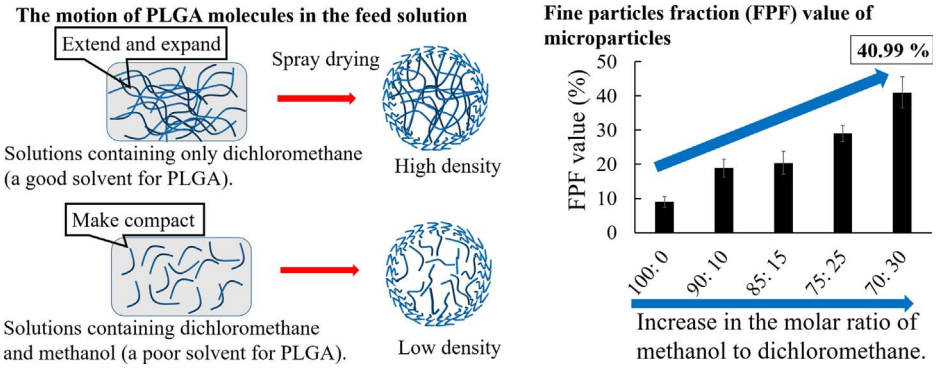
## GRAPHICAL ABSTRACT




## ARTICLE INFO

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## ABSTRACT

The purpose of this study was to reveal the effects of the conformation of poly (DL-lactide-*co*-glycolide) (PLGA) molecules in the feed solution on the aerodynamic diameter of PLGA microparticles prepared by using spray drying method. We investigated the conformation of PLGA molecules in the feed solution using viscometry. The data provide information about the polymer coil radius ($R_{coil}$), the overlap concentration ($c^*$). Then, we prepared various rifampicin-loaded PLGA microparticles by changing the mixing ratio of dichloromethane and methanol. We used a cascade impactor and mice to measure the aerodynamic diameter of the microparticles *in vitro* and *in vivo*, respectively. The viscosity measurement showed that an increased molar ratio of methanol in the solvent compositions resulted in the decreased $R_{coil}$ and increased $c^*$. Then, we found that the increased molar ratio of methanol in the solvent compositions resulted in the increased fine particle fraction value *in vitro* and delivery ratio to lung *in vivo*. The conformation of PLGA molecules in the feed solutions influences PLGA network in the microparticles, which would affect the aerodynamic diameter of the microparticles. In conclusion, the finding of our study suggests that solvent selection and connectivity of PLGA molecules of microparticles are associated with the aerodynamic diameter.

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## 1. Introduction

Poly (lactic-*co*-glycolic acid) (PLGA) has been widely used and studied of its biodegradability, biocompatibility, toxicological safety and release of drugs [1–4]. PLGA has also been used in studies of pulmonary drug delivery and treatment of tuberculosis [5–9]. Previously the authors have developed rifampicin (RFP)-loaded PLGA microparticles to kill Mycobacterium tuberculosis in alveolar macrophage [10,11]. However, it should also be pointed out that fine particle fraction (FPF) value of microparticles was very low (approximately 6%) [12]. It is needed to clarify the factors which influence aerodynamic diameter and make FPF value of particles higher. We hypothesize that the conformation of PLGA molecules in the organic solvent, feed solution, is important because the more compact PLGA molecular conformation would lead to having smaller aerodynamic diameter of microparticles. The aerodynamic diameter, $d_{aer}$, of the microparticles was calculated by the Eq. (1):

$$d_{aer} = d_{mass}\sqrt{\frac{\rho}{F}} \quad (1)$$

where $d_{mass}$, ρ and $F$ are the geometrical particle diameter, the density of particle and the shape fraction, respectively [7]. We focused on the density of particles to improve aerodynamic diameter of microparticles. Our hypothesis relies on the previous studies. The studies revealed that the conformation of PLGA molecules influences the connectivity of PLGA and affects the drug release kinetics. Further, the reports suggest the importance of optimal solvent selection in designing polymeric microparticles with controlled release properties using spray drying method. It is found that an extended PLGA molecular conformational structure in a good solvent causes a slow release rate, which might be due to a high density of the stable PLGA network formed during the spray drying process. In contrast, the more compact PLGA molecular conformation in a poor solvent results in a burst release, which might be attributed to the weaker and a low density of PLGA network formed during spray drying process [13,14]. Thus, we hypothesize that the conformation of PLGA molecules is also important for pulmonary drug delivery because low density of PLGA network causes FPF value of microparticles higher.

The purpose of this study was to clarify the effects of the conformation of PLGA molecules in the feed solution on the aerodynamic diameter of microparticles prepared using spray drying method. To examine the conformation of PLGA molecules in the feed solution, we performed viscosity measurement experiment. Then, we prepared RFP-loaded PLGA microparticles in mixtures of dichloromethane (DCM) and methanol (MeOH) at various ratio by using spray drying method. DCM was chosen as a proper solvent for PLGA [15] and MeOH was as a poor solvent [16]. It is easy to prepare microparticles using spray drying because boiling point of MeOH (62 °C) is lower than others. To investigate effects of the conformation of PLGA molecules in the feed solution on the aerodynamic diameter of spray dried microparticles, FPF value and delivery ratio to the lung were conducted *in vitro* and *in vivo*, respectively.

## 2. Materials and methods

### 2.1. Materials

PLGA with a molecular weight of 20,000 and a DL-lactic acid/glycolic acid monomer composition of 75/25 (PLGA7520), acetonitrile (for HPLC, purity ≥ 99.9%), dichloromethane (DCM, $CH_2Cl_2$, purity ≥ 99.0%), methanol (MeOH, $CH_3OH$, purity ≥ 99.8%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Rifampicin (RFP, $C_{43}H_{58}N_4O_{12}$, purity ≥ 97.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Table 1**
Characterization of PLGA molecules in the feed solution (n = 3).

| Solvent compositions (DCM: MeOH) | [η] (dL/g) | $R_{coil}$ (nm) | $V_{coil}$ ($nm^3$) | $c^*$ (g/dL) | Entanglement index |
|---|---|---|---|---|---|
| 100: 0 | 0.33 | 1.02 | 4.44 | 3.03 | 1.32 |
| 90: 10 | 0.32 | 1.00 | 4.19 | 3.14 | 1.27 |
| 85: 15 | 0.29 | 0.97 | 3.82 | 3.43 | 1.17 |
| 75: 25 | 0.24 | 0.92 | 3.26 | 4.10 | 0.98 |
| 70: 30 | 0.18 | 0.82 | 2.31 | 5.52 | 0.72 |

**Table 2**
Characterization of rifampicin-loaded PLGA microparticles (n = 3, mean ± S.D.).

| Solvent compositions (DCM: MeOH) | Mean volume diameter (μm) | Drug loading (%) | Entrapment efficiency (%) |
|---|---|---|---|
| 100: 0 | 4.71 ± 2.61 | 20.21 ± 0.08 | 101.1 ± 0.42 |
| 90: 10 | 4.98 ± 2.72 | 20.13 ± 0.07 | 100.6 ± 0.34 |
| 85: 15 | 4.47 ± 2.57 | 20.11 ± 0.18 | 100.5 ± 0.93 |
| 75: 25 | 4.65 ± 2.59 | 19.11 ± 0.21 | 99.55 ± 1.06 |
| 70: 30 | 4.37 ± 2.51 | 19.92 ± 0.02 | 99.62 ± 0.12 |

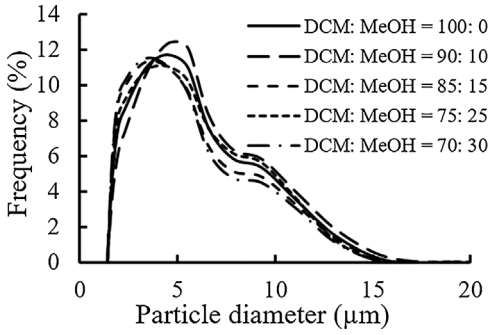


**Fig. 1.** Size distributions of microparticles prepared using various solvent compositions.

Hydroxypropyl methylcellulose (HPMC) capsules were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Other chemicals were of the highest grade commercially available.

### 2.2. Viscosity measurement of the feed solutions

In order to measure the conformation of PLGA molecules in the feed solution, viscometer (TV-20, Toki Sangyo Co., Ltd., Tokyo, Japan) was introduced. The experiment was carried out at 25 °C in a water bath because viscosity tends to affect the temperature [13]. The intrinsic viscosity ([η]) of PLGA in the various solvent compositions (DCM: MeOH = 100: 0, 90: 10, 85: 15, 75: 25, 70: 30) was determined by using the following Eq. (2):

$$[\eta] = \lim_{c\to 0}\left(\frac{\eta_{sp}}{c}\right) \quad (2)$$

where $\eta_{sp}$ is the specific viscosity and $c$ is the concentration of the polymer solution [17].

The polymer coil radius, $R_{coil}$, in the solvent compositions was calculated using [η] using Eq. (3):

$$R_{coil} = [3[\eta]\bullet M\mathrm{w}/10\pi\bullet N_{AV}]^{1/3} \quad (3)$$

where $M_w$ is the molecular weight of the polymer and $N_{AV}$ is Avogadro's number [18].

Volume per polymer coil, $V_{coil}$, in the various solvents was

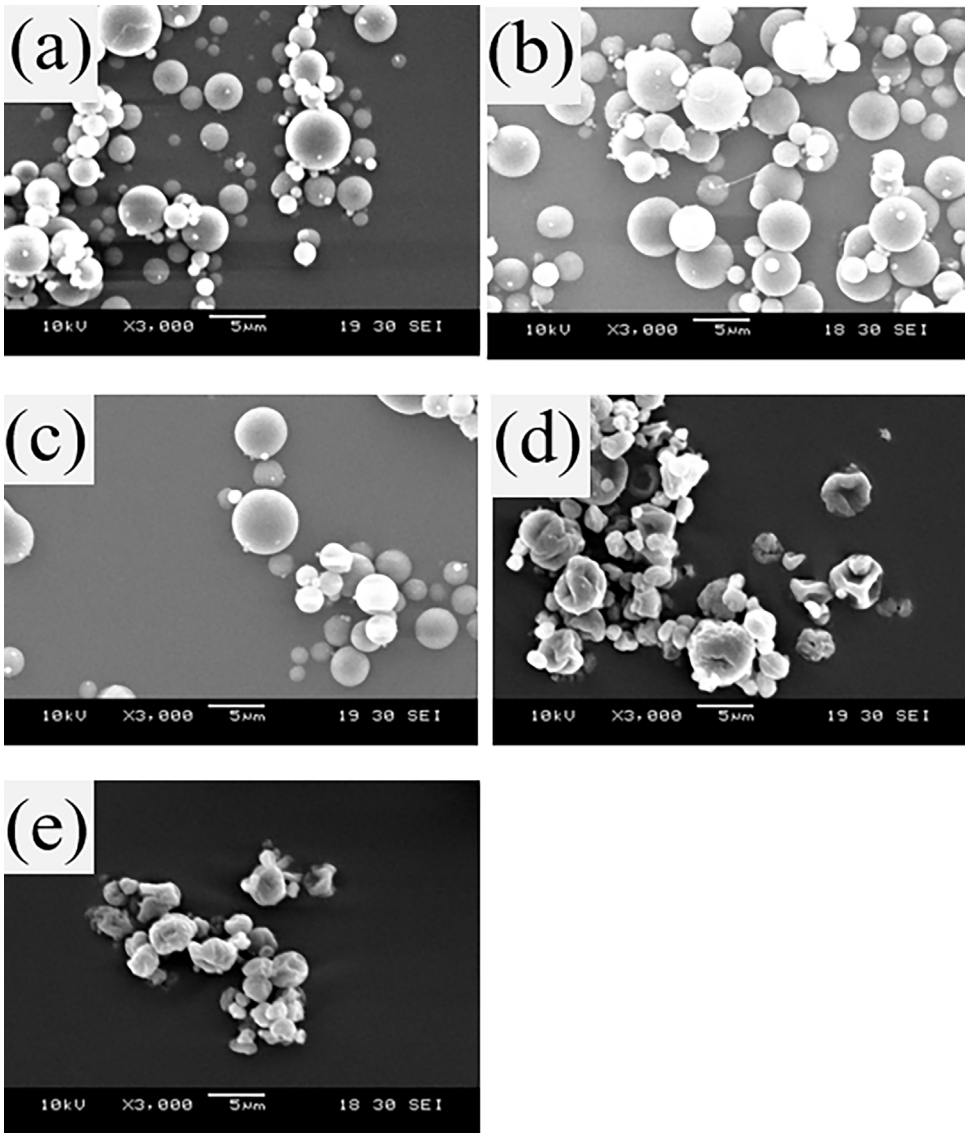


**Fig. 2.** Scanning electron microscope images of PLGA microparticles loaded rifampicin prepared using DCM: MeOH = 100: 0 (a), DCM: MeOH = 90: 10 (b), DCM: MeOH = 85: 15 (c), DCM: MeOH = 75: 25 (d), DCM: MeOH = 70: 30 (e) at an accelerating voltage of 12–14 kV (magnification: 3000 ×).

calculated using $R_{coil}$ by using Eq. (4) [18]:

$$V_{coil} = \left(\frac{4}{3}\right)\pi(R_{coil})^3 \quad (4)$$

Also, the overlap concentration ($c^*$) for PLGA in the various solvents was calculated using [$\eta$] using Eq. (5) [19]:

$$c^* = \frac{1}{[\eta]} \quad (5)$$

Furthermore, an entanglement index was calculated using $c^*$ by using Eq. (6):

$$\text{Entanglement index} = C_{PLGA}/c^* \quad (6)$$

where $C_{PLGA}$ is the polymer concentration in the feed solution [13].

### 2.3. Preparation of rifampicin-loaded PLGA microparticles

The PLGA microparticles loaded with RFP were prepared by using a spray dryer (B-290, BÜCHI Co., Ltd.). The spraying solutions were obtained by dissolving 400 mg of PLGA7520 and 100 mg of RFP in mixtures of DCM and MeOH at various ratio (DCM: MeOH = 100: 0, 90: 10, 85: 15, 75: 25, 70: 30). PLGA precipitated out at around DCM: MeOH = 65: 35. Spray drying was carried out under the following conditions, the outlet temperature of 37–40 °C, the air volume of 22.5 $m^3$/h, and pump flow rate of 2.5 mL/min. The mean volume diameter of microparticles in the air was measured by using a sizer (LDSA-3500A, Nikkiso Co., Ltd., Tokyo, Japan). RFP contents in the particles were measured using high-performance liquid chromatography (HPLC, SIL-20A prominence, SPD-20A prominence, LC-20AD prominence, CTO-10ASvp, DGU-20$A_3$ prominence, Shimadzu) at 254 nm with an ODS column (STR ODS-M, size: 4.6 nm × 150 nm, Shinwa Chemical Industries Ltd., Kyoto, Japan). Acetonitrile: Phosphate buffer solution (pH 2.6) = 3:2 was the mobile phase. The samples were dissolved in 10 mL of the solution. In addition, 5 mg of RFP were dissolved in 10 mL of the solution as a control. HPLC measurements were carried out at 40 °C (flow rate: 1 mL/min), and 50 μL of sample solution were applied. All HPLC measurements were carried out under the same conditions. The morphology of RFP- loaded PLGA microparticles were observed using a scanning electron microscope (SEM, JSM-6060LA, JEOL Ltd., Akishima, Japan).

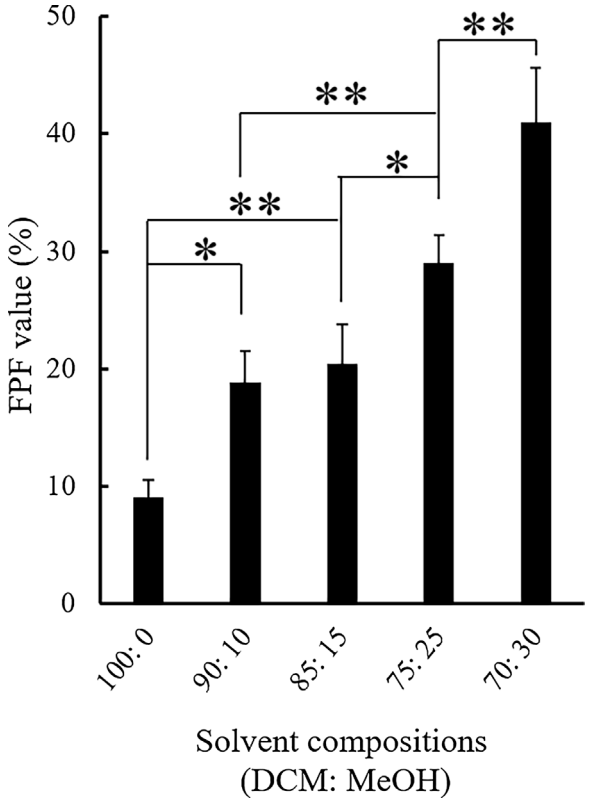


**Fig. 3.** FPF value of microparticles prepared by using various solvent compositions (mean ± S.D., n = 3, *$p < .05$, **$p < .01$, Tukey's test).

### 2.4. Measurement of the aerodynamic diameter of the microparticles

#### 2.4.1. In vitro measurement of FPF value

To evaluate the effects of the conformation of PLGA molecules in the feed solution on the aerodynamic diameter of spray dried microparticles, FPF value was measured using a cascade impactor (Andersen nonviable impactor MODEL AN-200, Tokyo Dylec Co., Ltd., Tokyo, Japan) with a dry powder inhaler (DPI) (Jethaler, Hitachi, Ltd., Tokyo, Japan). One HPMC capsule containing 5 mg of microparticles was settled in DPI, and DPI was attached to inlet of the cascade impactor [20]. The measurement was carried out at a steady flow rate of 28.3 L/min for 5 s at a relative humidity of 90%. A number of the particles left in each stage was measured using HPLC.

#### 2.4.2. Animal experiments

The purpose of this animal experiments is to confirm and support the results of the FPF value of the microparticles. Male ICR mice, approximately 8 weeks old were purchased from Japan Sankyo Labo. Service Corp. (Tokyo, Japan). Mice were anesthetized using isoflurane. And then, 5 mg of two kinds of microparticles (prepared by using DCM: MeOH = 100:0, the lowest FPF value of the other microparticles, and DCM: MeOH = 70:30, the highest FPF value) were administered to ICR mice using an animal DPI (Dry Powder Insufflator Model DP-4M, Penn Century, Inc., US). Furthermore, the mice were sacrificed by cervical dislocation, and the lungs were obtained. The lungs were washed by using physiological saline. The isolated lungs were cut into small parts to obtain fine pieces. Obtained tissues were suspended in physiological saline of 4 times volume. The suspension was homogenized by using a homogenizer (Physcotron Microhomogenizer NS-310E, Microtec Nition., Co., Ltd., Japan). Then, an appropriate amount of ethanol was added to homogenized tissues in order to remove protein. The lung tissue suspension was centrifuged at 1500 rpm for 5 min and then the supernatants of them were obtained. Finally, the supernatants were dried under nitrogen atmosphere and then dissolved in 3 mL of the mobile phase. The samples were analyzed using HPLC with the same conditions as mentioned above.

Mice care was conducted in accordance with the Guidelines for Animal Experimentation of Tokyo University of Science, which are based on the Guidelines for Animal Experimentation of Japanese Association for Laboratory Animal Science.

## 3. Results and discussion

### 3.1. Characterization of the feed solutions

The characterization of the feed solutions is shown in Table 1. The first point to be discussed was the PLGA conformational structure in the solvent. We obtained the following results: $R_{coil}$ and $V_{coil}$ decreased upon increasing the molar ratio of MeOH in the solvent compositions. $R_{coil}$ and $V_{coil}$ are used to estimate polymer conformation formed in the solvent compositions [19,21]. These results supported the findings of prior studies that the polymer molecules such as PLGA tend to swell and expand in a good solvent, whereas a more compact polymer structure is formed in a poor solvent [13,14]. These polymer conformational changes in the solvent influences $c^*$ and entanglement index. Looking at Table 1 you will see that $c^*$ increased and entanglement index decreased upon increasing the molar ratio of MeOH in the solvent compositions. It is reported that at very low polymer concentration, individual molecules are separated from each other by many solvent molecules. At increased polymer concentration, the space between random coils is reduced until the molecules come in contact, and eventually overlapping. The polymer concentration is defined as "the overlap concentration ($c^*$)". The entanglement index was used to assess the network of the polymer in the solvent compositions [13,14]. Findings strongly support previous studies.

### 3.2. Characterization of RFP-loaded PLGA microparticles

The mean volume diameter, the RFP loading, and entrapment efficiency are summarized in Table 2, and the size distributions of microparticles are shown in Fig. 1. The mean particle diameters of the microparticles prepared by using the various solvent compositions were between 4 and 5 μm. RFP loading and entrapment efficiency were approximately 20% and 100%, respectively. From the SEM images, near-spherical microparticles were observed (Fig. 2). We confirmed that the solvent compositions did not significantly influence the mean volume diameter, the RFP loading and entrapment efficiency. We think the fact that solvent compositions did not these factors is good because when these factors make a lot of changes, it is difficult to discuss the relationship between conformation of PLGA molecules and the aerodynamic diameter of microparticles.

### 3.3. Effects of the motion of PLGA molecules in the feed solution on the aerodynamic diameter of the microparticles

#### 3.3.1. In vitro measurement of the FPF value of the microparticles

FPF value and aerodynamic diameter of microparticles prepared by various solvents are shown in Figs. 3 and 4, respectively. As shown Fig. 3, the FPF value of the microparticles prepared with DCM and MeOH molar ratios of 100: 0, 90: 10, 85: 15, 75: 25 and 70: 30 were 9.02 ± 1.56, 18.88 ± 2.62, 20.41 ± 3.35, 29.01 ± 2.36, and 40.99 ± 4.58, respectively. In briefly, FPF value increased upon increasing the molar ratio of MeOH in the solvent compositions. As shown Fig. 4, aerodynamic diameter of microparticles tended to be smaller upon increasing the molar ratio of MeOH in the solvent compositions.

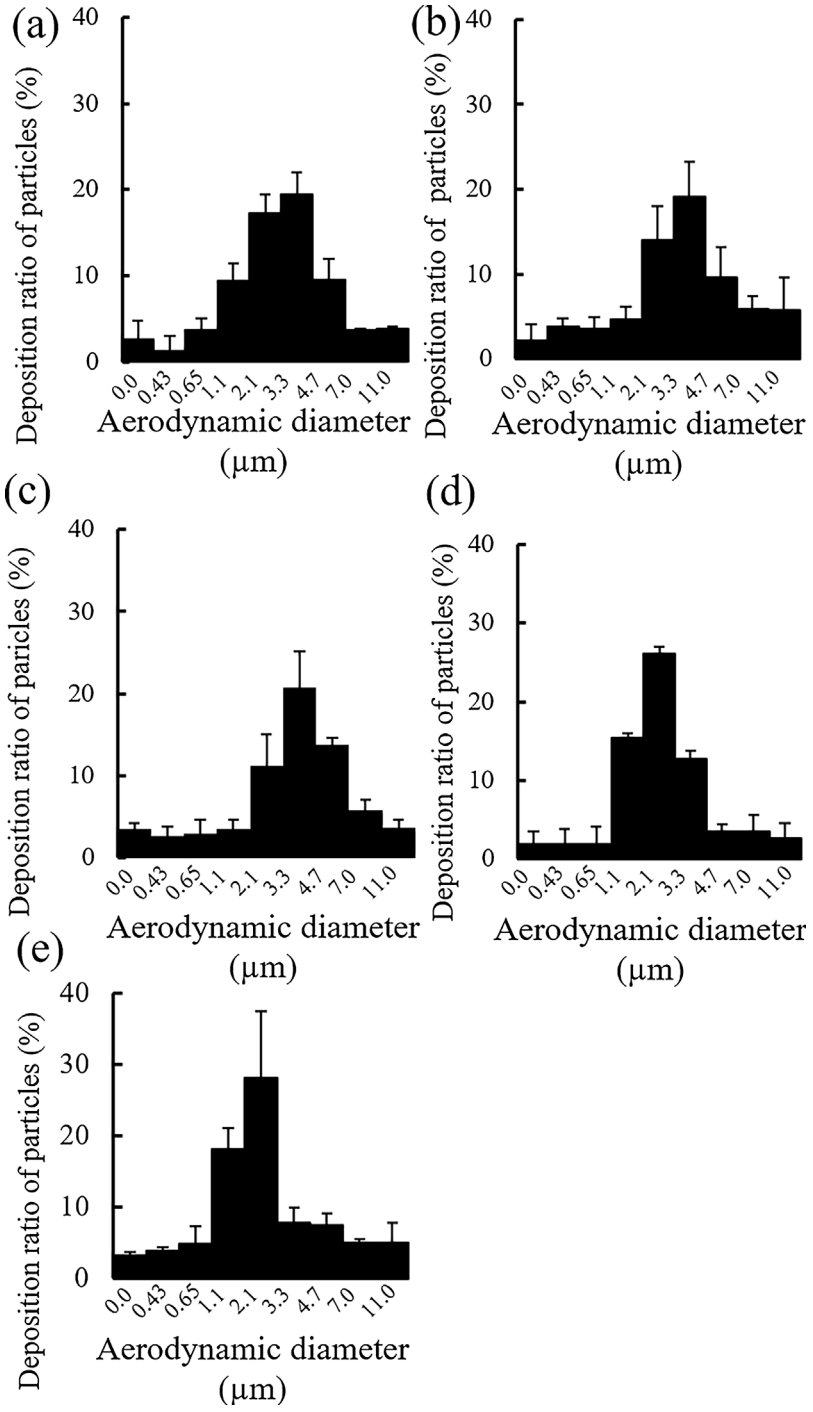


**Fig. 4.** Fraction distribution of aerodynamic diameter of microparticles prepared by using DCM: MeOH = 100: 0 (a), DCM: MeOH = 90: 10 (b), DCM: MeOH = 85: 15 (c), DCM: MeOH = 75: 25 (d), DCM: MeOH = 70: 30 (e) (mean ± S.D., n = 3).

*3.3.2. In vivo measurement of delivery ratio of microparticles to lung*

As shown Fig. 5, delivery ratio of microparticles prepared by molar ratio of DCM: MeOH = 70:30 to lung was significantly different from that of microparticles prepared by DCM: MeOH = 100:0 ($p < 0.01$). The results support the results of FPF value because the FPF value of the microparticles prepared with DCM and MeOH molar ratios of 100: 0 and 70: 30 were significantly different (Fig. 3). Further, as shown Fig. 6, deposition ratio of two kinds of microparticles was significantly different. The ratio of microparticles prepared by using DCM: MeOH = 70: 30 in lung was as twice as in trachea whereas the ratio of microparticles prepared by using DCM: MeOH = 100: 0 in lung as half as in trachea. The data means that aerodynamic diameter of microparticles has been smaller upon the increased molar ratio of MeOH.

As shown in Section 3.1, the PLGA molecules tend to extend in DCM, whereas they tend to make a compact structure in MeOH. Then, as shown in Section 3.3, aerodynamic diameter of microparticles tends to be smaller upon increasing molar ratio of MeOH. These results confirmed our hypothesis that the conformation of PLGA molecules in the feed solution influences PLGA network in the spray dried microparticles. The network would affect the aerodynamic diameter because the density of microparticles was changed. It is assumed that weaker network structure makes the low density of the microparticles [22]. The more compact conformation of PLGA in a poor solvent resulted in a weaker network formed during spray drying [13,14]. Therefore, it is

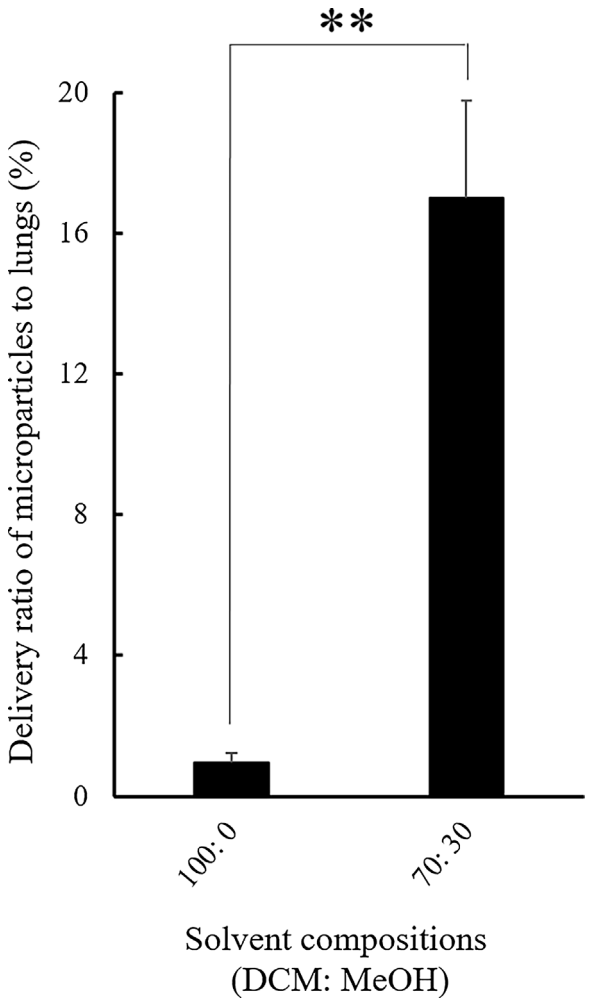


**Fig. 5.** Delivery ratio of microparticles prepared by using DCM: MeOH = 100: 0 (a) and DCM: MeOH = 70: 30 (b) to lung tissue by inhalation (mean ± S.D., n = 5, $^{**}p < .01$, Tukey's test).

obvious that the conformation of PLGA molecules in the feed solution is important to improve aerodynamic diameter. Solvent can have a great impact on the conformation of polymer [21,23,24], which would affect the properties of spray dried microparticles such as the aerodynamic diameter.

## 4. Conclusions

The strengths of this study are to introduce the concept of viscosity and reveal the importance of polymer conformation for pulmonary drug delivery. The study implies the conformation of PLGA molecules in the feed solution influences the connectivity and matrix of PLGA of spray dried microparticles, which affected aerodynamic diameter. From the results of viscosity measurement, we confirmed that conformation of PLGA molecules tended to be smaller upon increased the molar ratio of MeOH. Then, FPF value and animal experiment showed that aerodynamic diameter of microparticles has been smaller upon the increased molar ratio of MeOH. From these results, in a good solvent, an extended PLGA molecular conformational structure causes higher aerodynamic diameter because of higher density of PLGA network. In contrast, the more compact PLGA molecular conformation causes smaller aerodynamic diameter because of lower density. Therefore, it is obvious that solvent selection is important in the design of aerodynamic diameter of microparticles. This study constitutes a first step toward preparation of microparticles for pulmonary drug delivery using the concept of conformation of polymer.

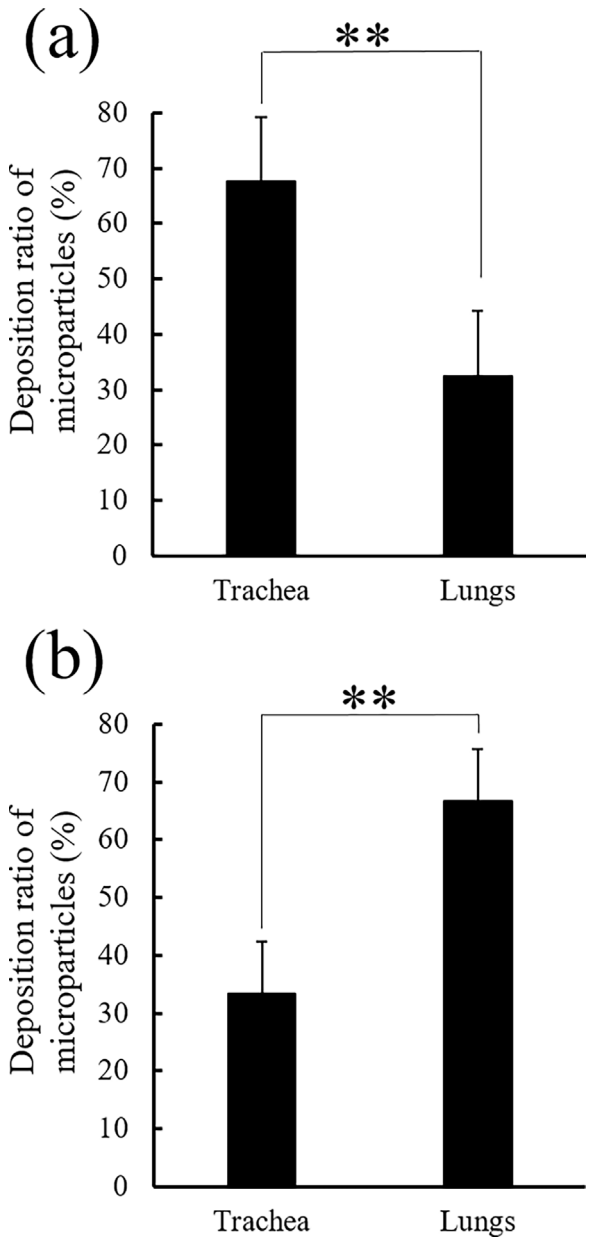


**Fig. 6.** Deposition ratio of microparticles prepared by using DCM: MeOH = 100: 0 (a), DCM: MeOH = 70: 30 (b) in lung and trachea (mean ± S.D., n = 5, $^{**}p < .01$, Tukey's test).

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