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Drug release behavior of hydrophobic drug-loaded poly (lactide-*co*-glycolide) nanoparticles: Effects of glass transition temperature

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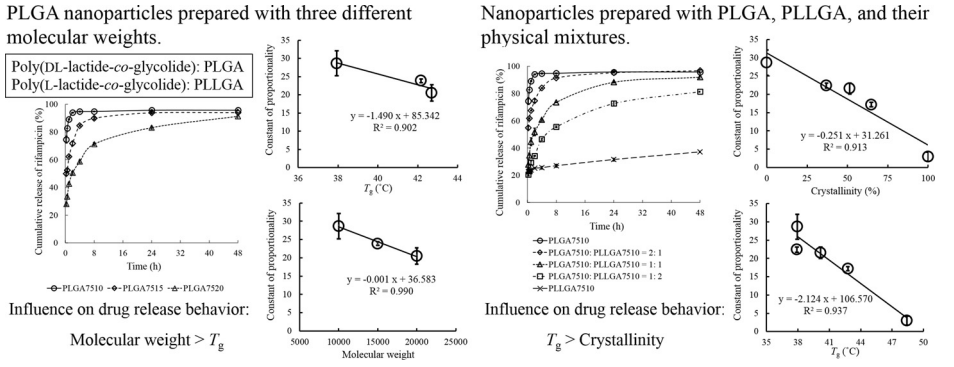
HIGHLIGHTS

- Poly(L-lactide-*co*-glycolide) of lactic acid/glycolic acid = 3/1 was synthesized.
- Physical mixtures of copolymers changed drug release behavior from nanoparticles.
- Influence of molecular weight on drug release behavior was greater than T_g.
- Influence of T_g was greater than crystallinity when molecular weights were same.

GRAPHICAL ABSTRACT



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ABSTRACT

Poly(lactide-*co*-glycolide) has been widely used and studied because of its biocompatibility and biodegradability. Recently, effects of physicochemical properties of poly(lactide-*co*-glycolide) on drug release behavior of hydrophobic drug-loaded nanoparticles were studied, and it was suggested that the glass transition temperature (T_g) of copolymers greatly affected the drug release. In this study, we prepared rifampicin-loaded nanoparticles with 180-nm diameter using poly(DL-lactide-*co*-glycolide) (PLGA), poly(L-lactide-*co*-glycolide) (PLLGA), and their physical mixtures. We determined the crystalline states, glass transition temperatures, and *in vitro* release studies were carried out in phosphate-buffered saline at 37 °C for 48 h. The results of the drug release studies of PLGA nanoparticles with three different molecular weights indicated that the molecular weight had a greater influence on the drug release behavior than T_g in the molecular weight range of 10,000–20,000. Also, from the results of release studies of nanoparticles prepared using PLGA7510, PLLGA7510, and their physical mixtures, it was suggested that the influence of T_g on drug release behavior was greater than that of crystallinity when the molecular weights were same and the T_g of the substances were sufficiently higher than the ambient temperature.

1. Introduction

In the field of drug delivery system, studies utilizing poly(lactide-*co*-glycolide), which is one of the classes of biodegradable polymers for pharmaceutical, are actively conducted due to their biocompatibility and biodegradability [1,2]. There are two types of poly(lactide-*co*-

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glycolide): Poly(DL-lactide-*co*-glycolide) (PLGA) and poly(L-lactide-*co*-glycolide) (PLLGA). These copolymers have been studied so far and their biocompatibility characteristics have been reported [3]. PLGA has been widely used and studied for transdermal administration and pulmonary drug delivery. PLGA nanoparticles for improving permeability of drugs to the skin and PLGA microsphere and nanocomposite particles for treatment of tuberculosis were reported [4–9]. Drug release from the formulation using poly(lactide-*co*-glycolide) has been studied. The degradation rate of PLGA increase, as the proportion of hydrophilic glycolic units increase [10]. An increase in the proportion of glycolic acid also induced rapid drug release from PLGA formulation [2,11]. Recently, by comparing rifampicin (RFP) release behavior from PLGA and PLLGA nanoparticles, similar characteristics were confirmed in PLLGA [12]. RFP, a semisynthetic bactericidal antibiotic drug, was used as a model drug. There are already many reports focused on using PLGA to prepare RFP-filled microspheres and nanoparticles [13–17]. It has been reported that the release of drug from nanoparticles involved an initial rapid release phase, which was followed by a relatively slow release phase. Generally, the mechanisms by which active agents can be released from a delivery system are the combination of diffusion of the active agent passes through the polymer that forms the controlled-release device, polymeric erosion, swelling, and degradation. The initial burst is related to drug type, drug concentration, and polymer hydrophobicity. When a polymer has higher hydrophobicity, it retains drug more strongly in the formulation, and drug diffusion from the formulation was reduced by decreasing the flow of water into the formulation [2,12,18]. In addition, the glass transition temperature (T_g) of copolymers greatly affected the release behavior of RFP from PLGA and PLLGA nanoparticles with diameters of 100, 200, and 400 nm. In this study, it was suggested that RFP within the nanoparticles was also released under the temperature condition close to T_g of poly(lactide-*co*-glycolide) since the fluidity of the copolymers increased; this indicated that the T_g of copolymers greatly contributed to the initial burst from the drug-loaded nanoparticles at body temperature. [12].

In this study, we prepared RFP-loaded nanoparticles with PLGA, PLLGA, and their physical mixtures and measured physicochemical characteristics of copolymers to investigate their influences on release behavior of drug-loaded nanoparticles. To compare the influence of T_g and molecular weight of PLGA on RFP release behavior, nanoparticles were prepared from PLGA with different molecular weights. Also, nanoparticles were prepared from various physical mixtures of PLGA and PLLGA to investigate the controllability of RFP release behavior from nanoparticles by mixing PLGA and PLLGA and the influence of their mixing ratio on RFP release behavior.

2. Materials and methods

2.1. Materials

PLGA (monomer composition of DL-lactic acid/glycolic acid = 75/25) having molecular weights of 10,000 (PLGA7510), 15,000 (PLGA7515), and 20,000 (PLGA7520) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyvinyl alcohol (PVA, degree of polymerization: 500), trehalose dihydrate ($C_{12}H_{22}O_{11}{\cdot}2H_2O$, purity ≥ 98%), tin (II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$, purity ≥ 97%) and chloroform-d ($CDCl_3$, purity ≥ 99.8%, containing 0.05 vol% TMS) were also purchased from Wako Pure Chemical Industries, Ltd. L-lactic acid ($CH_3CH(OH)COOH$, purity = 90-92%) was purchased from Musashino Chemical Laboratory, Ltd. (Tokyo, Japan). Glycolic acid ($C_2H_4O_3$, purity ≥ 98%) was purchased from Nacalai Tesque Inc. (Kyoto, Japan). RFP ($C_{43}H_{58}N_4O_{12}$, purity ≥ 97%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of the highest grade commercially available.

2.2. Synthesis of PLLGA7510

L-lactic acid and glycolic acid were mixed at a molar ratio of 3: 1 in a three-necked flask under nitrogen atmosphere. After stirring for 6 h at 140 °C and 4 kPa, a 4% (w/w) of tin (II) chloride dihydrate was added. The mixture was reacted at 165 °C and 1 kPa until its molecular weight became 10,000, which was measured using gel permeation chromatography (GPC). This polymer was dissolved in chloroform and purified by dropwise addition to diethyl ether. GPC (SIL-20A prominence, LC-20AD prominence, RID-10A, CTO-10ASvp, Shimadzu Co., Kyoto, Japan) was performed in chloroform with GPC columns (GPC K-803 and K-806, Showa Denko K. K., Tokyo, Japan). Molecular weights were calculated using polystyrene as the standard.

2.3. Measurement and characterization of copolymers and their physical mixtures

The ^{1}H NMR spectra were recorded in $CDCl_3$ on an FT-NMR system (NNM-AL400, JEOL Ltd., Akishima, Japan). The solid-state forms of copolymers were confirmed by XRPD (RINT-Ultima 3, Rigaku Co., Ltd., Akishima, Japan). The XRPD measurements were carried out in the standard measurement mode in the 2θ range from 10° to 50°. The scan speed was 2°/min and the counting step was 0.02°. X-ray source was CuKα, the accelerating voltage was 40 kV, and the current was 40 mA. Samples ground with a mortar and pestle were filled in a glass sample plate (0.2 mm depth). The T_g values of the copolymers were measured using differential scanning calorimeter (DSC8230, Rigaku Co., Ltd., Akishima, Japan). DSC measurements were conducted in crimped aluminum sample pans under 50 mL/min N_2 purge. Ten-milligram aliquots of samples in dry power state were measured at 15–153 °C with a heating rate of 1 °C/min. The T_g values were determined as the glass transition midpoint in obtained signal.

2.4. Preparation of RFP-loaded nanoparticles

RFP-loaded nanoparticles were prepared by using emulsion solvent evaporation method [5,7]. Briefly, 900 mg of copolymers and 100 mg of RFP were dissolved in 20 mL of dichloromethane. The solution was added to 100 mL of 2.0% (w/v) PVA aqueous solution and was emulsified using a probe sonicator (Digital Sonifier S-250D, Branson Ultrasonics Corp., Danbury, CT) at 200 W of energy output. The sonication was carried out for 90 s in an ice bath. Prepared O/W emulsion was stirred overnight on a magnetic stir plate at room temperature to evaporate dichloromethane. The nanoparticles were collected by ultracentrifugation at 15,000 rpm for 10 min (Himac 80WX, Hitachi Koki Co. Ltd., Tokyo, Japan). After centrifugation, the precipitated nanoparticles were washed with purified water in ultrasonic bath sonicator to remove residual PVA. This centrifugation and wash handling were repeated two times. The precipitation of nanoparticles was redispersed in purified water, and trehalose dihydrate with an equal weight of precipitated nanoparticles was added. After freezing at − 30 °C, this suspension was dried using freeze dryer (FD-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan).

Surface properties of nanoparticles were observed using scanning electron microscope (SEM, JSM-6060LA, JEOL Ltd., Akishima, Japan). The mean volume diameter and size distribution of the prepared RFP-loaded nanoparticles were measured using a particle size analyzer (ELSZ-2, Otsuka Electronics Co., Ltd., Osaka, Japan). RFP content in the prepared particles were measured using high-performance liquid chromatography (HPLC, SIL-20A prominence, SPD-20A prominence, LC-20AD prominence, CTO-10ASvp, DGU-20A_3 prominence, Shimadzu Co., Kyoto, Japan) at 254 nm with an ODS column (STR ODS-M, size: 4.6 mm–150 mm, Shinwa Chemical Industries Ltd., Kyoto, Japan). A mobile phase was the mixed solution of phosphate buffer (pH 2.6) and acetonitrile with a volume ratio of 2: 3. The samples were dissolved in 10 mL of the solution. Also, 30 mg RFP was dissolved in 10 mL of the

solution as a control. HPLC measurement was carried out at 40 °C (flow rate of 2.0 mL/ min) and 50 μL of the sample solution was applied. All HPLC measurements were carried out under the same conditions.

2.5. Release of RFP from nanoparticles

To evaluate the influence of poly(lactide-*co*-glycolide) composition on drug release behavior, release rates of RFP from RFP-loaded poly (lactide-co-glycolide) nanoparticles were studied. Fifteen milligrams of RFP-loaded nanoparticles were redispersed in 5 mL of phosphate-buffered saline (PBS). The sample solutions were shaken at 30 rpm at 37 °C. After 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 24.00, and 48.00 h, the samples were centrifuged at 15,000 rpm for 10 min (Himac 80WX) [19]. Precipitates were collected and dissolved in 3.0 mL of mobile phase. For determining released RFP concentration, HPLC measurement was carried out.

3. Results and discussion

3.1. Evaluation of copolymers and their physical mixtures

Copolymers were measured using ^{1}H NMR, GPC, XRPD and DSC. The basic chemical structure of PLLGA7510 was confirmed by ^{1}H NMR. As shown in Fig. 1a, one of the striking feature is a large peak at 1.5 ppm, corresponding to the methyl group of the lactic acid. The multiplets at 4.6–4.9 ppm and 5.1–5.2 ppm correspond to the glycolic acid CH_2 and the lactic acid CH, respectively [20–23]. The other peaks are those of diethyl ether used for purification. From the results of their integrated intensity ratio, the monomer composition of PLLGA7510 was determined as lactic acid/glycolic acid = 75.3/24.7. Also, the molecular weight of PLLGA7510 was 10,317. XRPD measurement results are shown in Fig. 1b. PLLGA7510 and physical mixtures of PLGA7510 and PLLGA7510 had higher crystallinity than PLGA groups. The degree of crystallinity of a substance greatly influences its solubility [24,25]. The T_g values of copolymers and their physical mixtures were calculated using DSC measurement results, and they are shown in Table 1. In the physical mixtures of PLGA7510 and PLLGA7510, T_g increased as the proportion of PLLGA increased. In PLGA with different molecular weight, T_g increased with increasing molecular weight, however, there was no significant difference between PLGA 7515 and PLGA 7520. These results are explained by the free volume model of polymer solids since the early 1950's. The free volume is greatly influenced by the crystalline state and the movement of the molecular chain ends, and the T_g increases due to increase in the degree of crystallinity or decrease in the number of end groups caused by an increase in polymerization degree [26–28].

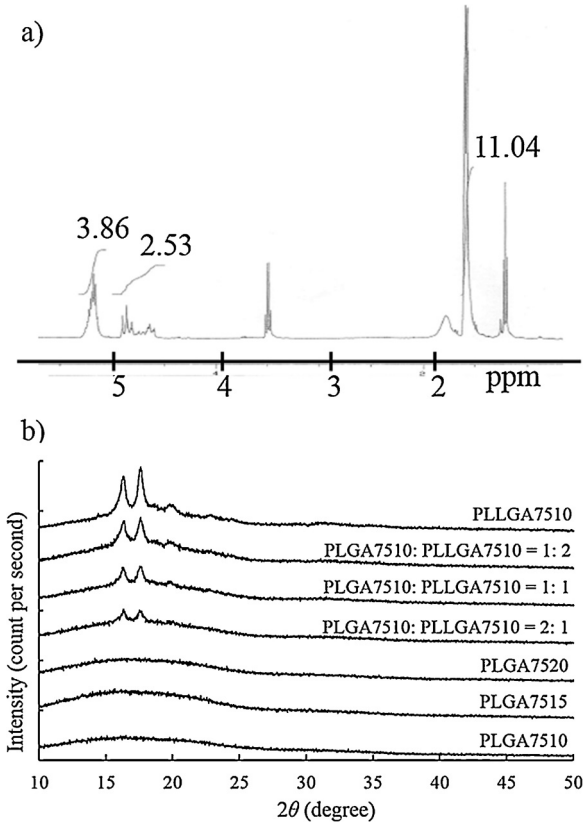

Fig. 1. a) ^{1}H NMR spectrum of synthesized PLLGA7510. b) X-ray diffraction patterns of PLGA7510, PLLGA7510, and their physical mixtures.

Table 1
Glass transition temperatures (T_g) of copolymers and their mixtures (n = 3, mean ± SD).

Composition of copolymers	T_g (°C)
PLGA7510	37.9 ± 0.3
PLGA7515	42.2 ± 0.1
PLGA7520	42.7 ± 0.2
PLGA7510: PLLGA7510 = 2: 1	37.9 ± 0.3
PLGA7510: PLLGA7510 = 1: 1	40.2 ± 0.1
PLGA7510: PLLGA7510 = 1: 2	42.8 ± 0.5
PLLGA7510	48.4 ± 0.4

Table 2
Properties of various rifampicin loaded nanoparticles (n = 3, mean ± SD).

Drug carrier	Mean volume diameter (nm)	Coefficient of variance	Rifampicin content (w/w%)
PLGA7510	182.7 ± 61.6	0.34	4.29 ± 0.01
PLGA7515	186.8 ± 56.3	0.30	3.68 ± 0.08
PLGA7520	188.3 ± 57.5	0.31	3.15 ± 0.05
PLGA7510: PLLGA7510 = 2: 1	186.2 ± 54.3	0.29	3.81 ± 0.02
PLGA7510: PLLGA7510 = 1: 1	188.8 ± 53.7	0.28	3.54 ± 0.01
PLGA7510: PLLGA7510 = 1: 2	187.0 ± 54.9	0.29	3.42 ± 0.01
PLLGA7510	184.7 ± 57.0	0.31	3.04 ± 0.02

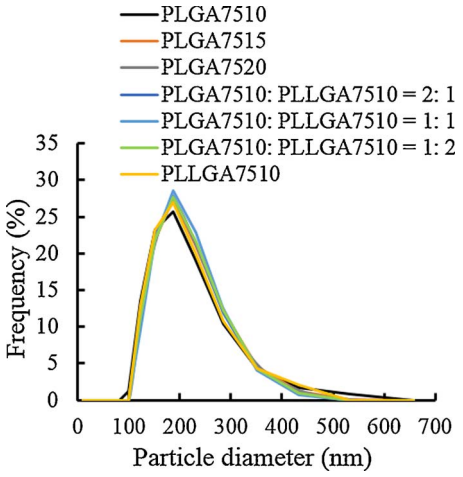

Fig. 2. Particle size distribution of nanoparticles prepared with PLGA7510, PLGA7515, PLGA7520, PLLGA7510, and their physical mixtures.

3.2. Characterization of RFP-loaded nanoparticles

The mean volume diameter, the coefficient of variance, and RFP content in nanoparticles of each sample were summarized in Table 2.

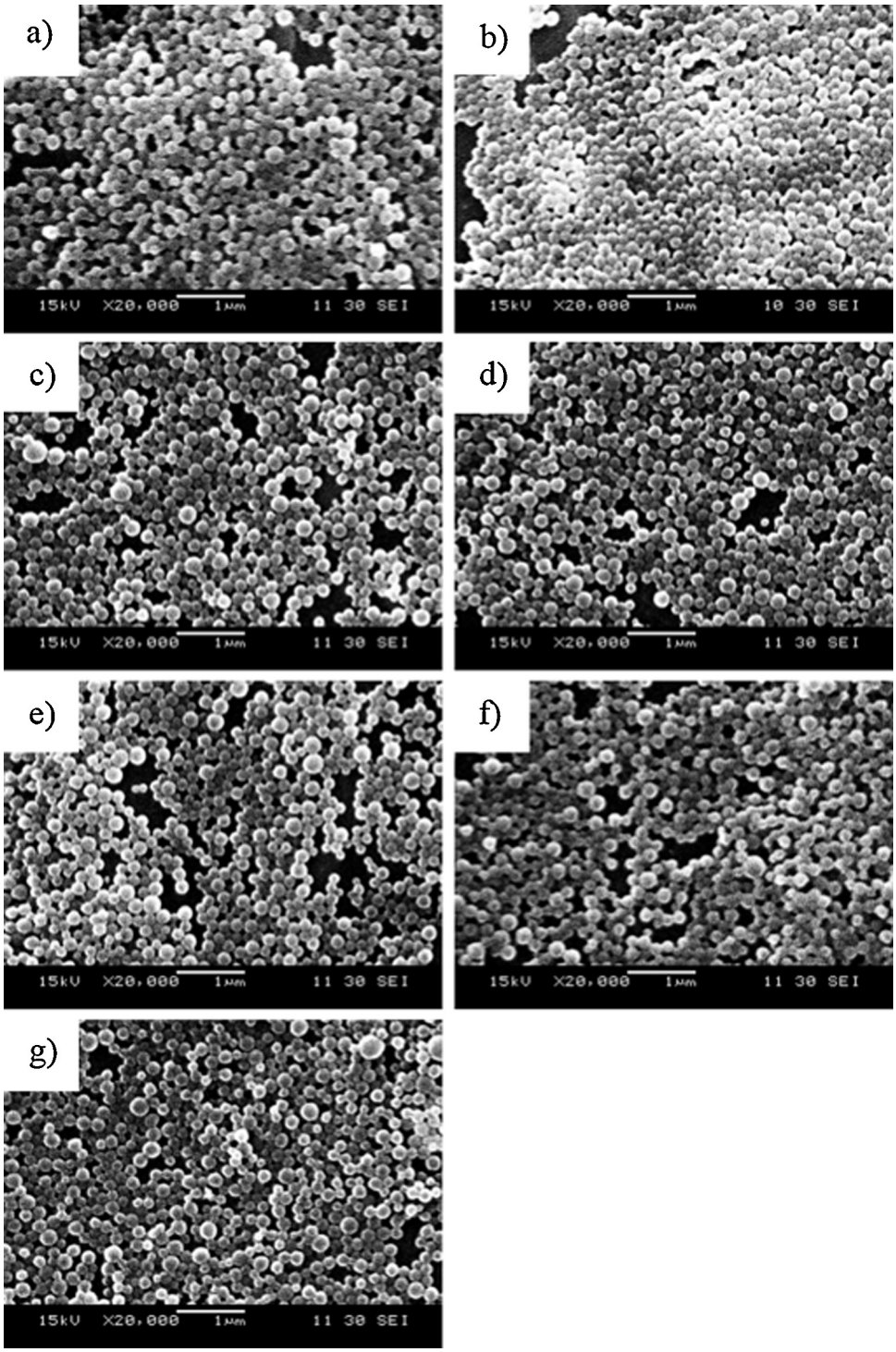

Fig. 3. Scanning electron microscopy images of rifampicin-loaded nanoparticles taken at an accelerating voltage of 15 kV and magnification of 10,000×. a) PLGA7510 nanoparticles. b) PLGA7515 nanoparticles. c) PLGA7520 nanoparticles. d) Nanoparticles prepared using physical mixtures with a 2: 1 polymer composition of PLGA7510: PLLGA7510. e) Nanoparticles prepared using physical mixtures with a 1: 1 polymer composition of PLGA7510: PLLGA7510. f) Nanoparticles prepared using physical mixtures with a 1: 2 polymer composition of PLGA7510: PLLGA7510. g) PLLGA7510 nanoparticles.

The mean volume diameters of prepared nanoparticles were 180–190 nm. The coefficient of variances of them, defined as the ratio of standard deviation to average diameter, were in the range of 0.25–0.35, and RFP content in nanoparticles were 3.0–4.3%. The influence of the difference of drug carrier on the content rate of RFP to nanoparticles showed the same tendency as the T_g measurement results. In the nanoparticles prepared using PLGA7520 and PLLGA7510, it was considered that the decrease in drug content occurred due to the decrease in free volume caused by the increase in crystallinity and molecular weight [26–28]. Fig. 2 shows size distribution of various nanoparticles. No significant differences in particle size distributions were observed between the nanoparticles. As the SEM images provided, the nanoparticles had a spherical shape (Fig. 3).

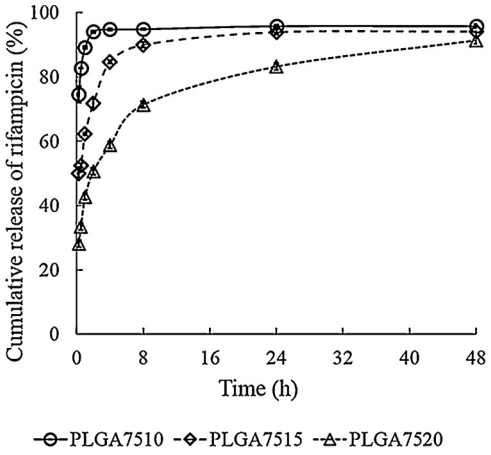

Fig. 4. Cumulative release rate of rifampicin from PLGA nanoparticles (n = 3, mean ± SD).

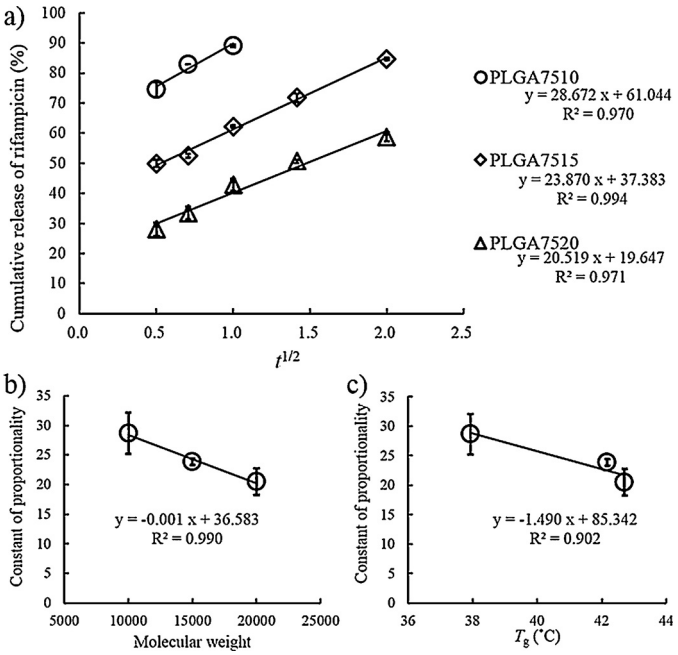

Fig. 5. a) The cumulative rifampicin release from PLGA nanoparticles against the square root of time t calculated using Higuchi's square root equation. b) The relationship between constant of proportionality and molecular weight. c) The relationship between constant of proportionality and glass transition temperature T_g (c) (n = 3, mean ± SD).

3.3. In vitro release of RFP from nanoparticles

The release rate of RFP from nanoparticles prepared using PLGA7510, PLGA7515 and PLGA7520 in PBS at 37 °C are shown in Fig. 4. As the molecular weight of PLGA increased, the cumulative release of RFP was decreased. Although it was suggested that the release behavior of drug from nanoparticles was greatly affected by T_g [12], comparing PLGA7515 nanoparticles and PLGA7520 nanoparticles, despite their very small difference in T_g, their release behavior had a significant difference. Therefore, we assumed that the influence of entanglement of PLGA molecular chains in nanoparticles on drug release behavior was greater than that of T_g. To verify this assumption, processing was carried out using the Higuchi's model to the part where the rapid release was observed (PLGA7510 nanoparticles were 0.25–1 h, PLGA7515 and PLGA7520 nanoparticles were 0.25–4 h). In this time range, the influence of swelling and corrosion of the nanoparticles was considered to be small because the size of the PLGA nanoparticles was maintained after 8 h of incubation [5]. Higuchi's square root equation is expressed as

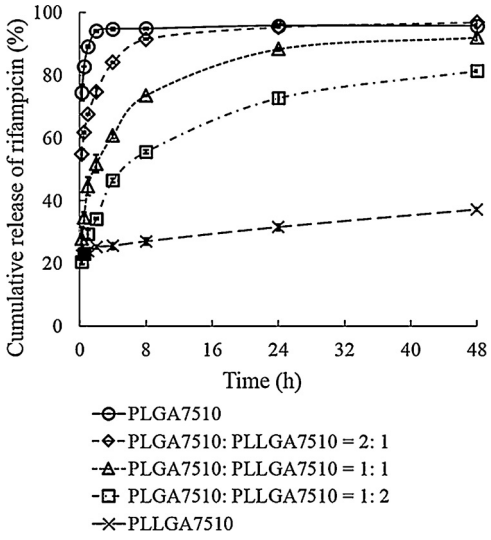

Fig. 6. Cumulative release rate of rifampicin from nanoparticles prepared with PLGA7510, PLLGA7510, and their physical mixtures (n = 3, mean ± SD).

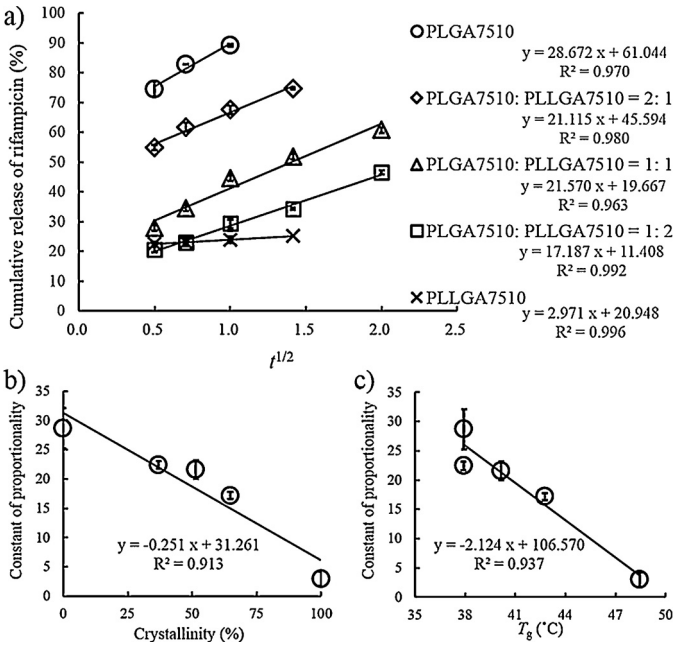

Fig. 7. a) The cumulative rifampicin release from nanoparticles prepared with PLGA7510, PLLGA7510, and their physical mixtures against the square root of time t calculated using Higuchi's square root equation. b) The relationship between constant of proportionality and crystallinity. c) The relationship between constant of proportionality and glass transition temperature T_g (n = 3, mean ± SD).

$$M_t = K_H t^{1/2},$$

where M_t is the amount of drug released at time t and K_H is the Higuchi rate constant [29].

The results are shown in Fig. 5a. In addition, as shown in Fig. 5b and c, figures were created in which the vertical axis was the slope as a proportionality constant of Higuchi's equation and the molecular weight or T_g was taken on the horizontal axis, and the correlations were evaluated using a general method of linear regression analysis [30]. From the comparison of Fig. 5b and c, a higher correlation was obtained in Fig. 5b. Therefore, it was suggested that the molecular weight of PLGA nanoparticles had more influence on drug release behavior than their T_g values.

The release rates of RFP from nanoparticles prepared using PLGA7510, PLLGA7510 and their physical mixture in PBS at 37 °C are shown in Fig. 6. As the previous study reported, RFP release from nanoparticles was greatly

prevented by using PLLGA 7510 when molecular weights of copolymers were the same [12]. Also, we could change the drug release behavior even with physical mixtures of PLGA7510 and PLLGA7510. However, in nanoparticles prepared using PLGA7510 and the physical mixture with a 2: 1 polymer composition of PLGA7510: PLLGA7510 with the same T_g between, a change in release behavior was observed. From the free volume model, this result was explained that differences in drug release behavior were caused by differences in crystalline states because compared with PLGA 7510 which is an amorphous state, the movement of the molecular chain was limited in the mixture having crystallinity [24–26]. From these results, we assumed that the influence of crystallinity of copolymers and their physical mixtures on drug release behavior were greater than that of T_g. To verify this assumption, processing was carried out using the Higuchi's model to the part where rapid release was observed (PLGA7510 nanoparticles were 0.25–1 h, nanoparticles prepared using PLLGA7510 and the physical mixture with a 2: 1 polymer composition of PLGA7510: PLLGA7510 were 0.25–2 h, nanoparticles prepared using physical mixtures with 1: 1 and 1: 2 polymer composition of PLGA7510: PLLGA7510 were 0.25–2 h). The analysis using the Higuchi's square root equation was also performed in the same manner as described above. The results are shown in Fig. 7a. In addition, as shown in Fig. 7b and c, figures were created in which the vertical axis was the slope as a proportionality constant of Higuchi's equation and the crystallinity or T_g was taken on the horizontal axis, and the correlations were evaluated using a general method of linear regression analysis. From the comparison of Fig. 7b and c, a higher correlation was obtained in Fig. 7b, however, in the sample with T_g close to the temperature of release test, the correlation was low. The influence of crystallinity was lower than expected, and this result might be due to the mixing of two different copolymers, PLGA7510 and PLLGA7510. Therefore, it was suggested that T_g of nanoparticles prepared using PLGA7510, PLLGA7510 and their physical mixtures had more influence on drug release behavior than their crystallinities, when copolymers and their physical mixtures had same molecular weight and had sufficiently higher T_g than the ambient temperature.

4. Conclusions

In vitro release behavior of RFP from PLGA nanoparticles with a molecular weight of 10,000–20,000 was studied, and it was suggested that in the relatively low molecular weight region, the influence of molecular weight on drug release behavior was greater than that of T_g. It is widely known that molecular weight affects drug release behavior from micro- and nanoparticles. Since the temperature of the release tests was lower than the T_g of all PLGA used and the difference in T_g was 4.8 °C at the maximum, we considered that the influence of T_g was relatively decreased. *In vitro* release behavior of RFP from nanoparticles prepared with PLGA7510, PLLGA7510 and their physical mixtures were also studied, and we confirmed that the release behavior of nanoparticles could be changed by mixing PLGA7510 and PLLGA7510. Moreover, it was revealed that the influence of T_g on drug release behavior was greater than that of crystallinity when molecular weights were same. In this study, T_g of PLLGA7510 was 11.4 °C higher than the ambient temperature. Thus, we considered that the influence of T_g was relatively increased. These results indicate that in addition to the molecular weight and crystallinity of copolymers, it is necessary to take into account the difference between their T_g and the surrounding temperature to control the release of the drug from the nanoparticles prepared with PLGA, PLLGA, and their physical mixtures. In addition, further investigation of drug release behavior is needed, especially when the T_g of a copolymer is close to the ambient temperature.

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