Effects of Polyvinyl Alcohol on Drug Release from Nanocomposite Particles Using Poly (L-lactide-co-glycolide)

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Abstract: The effects of polyvinyl alcohol (PVA) on the release behavior of polymer nanoparticles from nanocomposite particles using amino acids were investigated. Rifaximin (RFX) was used as a hydrophobic drug model. RFX-loaded poly(L-lactide-co-glycolide) (PLLGA) nanoparticles were prepared using an antisolvent diffusion method. They were then spray-dried with equal amounts of amino acids to prepare the nanocomposite particles. The mean diameters of nanocomposite particles were 2.86–5.42 μm . The particle size increased as the concentration of PVA aqueous solution increased. The mean diameters of RFX-loaded PLLGA nanoparticles were 150–160 nm; however, the particle size distributions of those prepared using 0.25% (w/v) PVA aqueous solution differed significantly immediately after preparation and after redispersion from nanocomposite particles. The release test results of nanocomposite particles revealed that those prepared using 0.25% and 0.50% (w/v) aqueous PVA solutions rapidly released RFX. In contrast, particles prepared using 2.00 and 4.00% (w/v) PVA aqueous solution showed sustained drug release. The results of drug release tests of nanoparticles redispersed from nanocomposite particles showed that the nanoparticles prepared using 0.50% and 2.00% (w/v) PVA aqueous solution suppressed the initial burst. Therefore, we considered that the results of the drug release behavior of the nanoparticles in these particles reflects the release behavior of the nanoparticles from the nanocomposite particles. These results indicate that the rate of redispersion from nanocomposite particles to nanoparticles can be controlled by changing the concentration of PVA aqueous solution.

Key words: poly(L-lactide-co-glycolide), nanoparticles, nanocomposite particles, polyvinyl alcohol, amino acids

1 Introduction

Parenteral drug administration has attracted attention in drug delivery systems. Bioavailability can be improved by avoiding the hepatic first-pass effect, which can prematurely metabolize drugs^{1,2)}. Inhalation systems are one of the non-invasive and self-administering methods. Inhalation formulations are a promising system for systemic administration because of the large surface area of the lungs and thin absorption barrier³⁾. In topical administration for the treatment of pulmonary diseases, they can deliver drugs directly and rapidly to the diseased lung tissue site, which is advantageous over oral formulations in which only a small proportion is delivered to the diseased site⁴⁾. To efficiently deliver the drug to the target site, it is necessary to control the size and shape of the inhalation formulation. It has been reported that particles with a mean aerody-

namic diameter of 1–3 μm are deposited minimally in the mouth and throat and maximally in the lung parenchymal regions, such as the alveolar or deep-lung region⁵⁾. Thus, inhalable nanocomposite particles are a promising inhalation system. Furthermore, nanoparticles can overcome mucus clearance⁶⁾. In addition to macrophages, they are also taken up by other cells such as cancer cells and epithelial cells^{7–10)}. These properties of nanoparticles are useful for drug delivery; however, the size of nanoparticles is not suitable for inhalation. Nanocomposite particles with an aerodynamic diameter suitable for inhalation are used to solve this problem. Since these particles use diluents soluble in the alveoli lining fluid, they are decomposed into nanoparticles after reaching the alveoli¹⁰⁾. Recently, it was reported that nanocomposite particles for inhalation improved the concentration of drug in the lungs. These

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studies with nanocomposite particles containing sildenafil¹¹⁾ and rifampicin¹²⁾-loaded nanoparticles demonstrate the usefulness of transpulmonary administration using polymer nanoparticles. Polymer nanoparticles are useful carriers because of their biocompatibility, biodegradability, ease of surface modification, localization, and reduced systemic toxicity¹³⁾. In addition, they are capable of controlling drug release behavior. In previous studies, we investigated the physicochemical properties that affect the drug release behavior of nanoparticles prepared using poly(lactide-co-glycolide)^{14, 15)}. In studies using curcumin⁴⁾, tadalafil¹⁶⁾, and simvastatin¹⁷⁾, nanocomposite particles containing drug nanoparticles were prepared using the spray drying method. It was reported that nanocomposite particle formation increased solubility, mainly due to the amorphization of the drug. Studies have been conducted on the behavior of drug release from nanocomposite particles, whereas there are few studies on the behavior of the release of nanoparticles from them. While sugars have conventionally been used as diluents for nanocomposite particles, amino acids have recently attracted attention for improving pulmonary delivery in high humidity^{18–20)}. In general, amino acids have a lower solubility in water than sugars. Hence, research focusing on their dissolution behavior is necessary.

In this study, we controlled the release behavior of poly(L-lactide-co-glycolide) (PLLGA) nanoparticles from nanocomposite particles using polyvinyl alcohol (PVA). PVA is used in various pharmaceutical applications, such as stabilizing agents for emulsions²¹⁾, artificial tears²²⁾, and sustained-release formulations for oral administration²³⁾. An evaluation of oral toxicity showed that this additive was safe even at high levels of consumption²⁴⁾. The nanoparticle surfaces are easily coated with PVA using an aqueous PVA solution²⁵⁾. We considered that the PVA-derived hydrated layer on the nanoparticles' surface caused an efficient influx of water into the nanocomposite particles and aided in the redispersion of the nanoparticles. PLLGA was suitable for this study because it can prepare nanoparticles with slower drug release than those using PLGA^{14, 15)}. Rifaximin (RFX), a hydrophobic antibacterial agent, was used as a model drug²⁶⁾. Release tests were performed on the nanocomposite particles to investigate nanoparticles' release behavior after reaching the lungs. Similarly, release tests were performed on nanoparticles redispersed from nanocomposite particles to investigate drug release behavior after reaching the lungs. The relationship between nanoparticles, nanocomposite particles, and nanoparticles redispersed from the nanocomposite particles is shown in Fig. 1.

Fig. 1 Schematic diagram of the relationship between nanoparticles, nanocomposite particles, and nanoparticles redispersed from nanocomposite particles.

2 Experimental Procedures

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). RFX ($C_{43}H_{51}N_3O_{11}$, purity $\geq 98.0\%$), polyvinyl alcohol (PVA, degree of polymerization: 500), ethanol (EtOH, purity $\geq 99.5\%$), acetone (CH_3COCH_3 , purity $\geq 99.5\%$), L-(+)-arginine hydrochloride (purity $\geq 99.0\%$), and L-leucine (purity $\geq 99.0\%$) were purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). Acetonitrile (CH_3CN , for HPLC, purity $> 99.9\%$) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

2.2 Preparation of RFX-loaded PLLGA nanoparticles

RFX-loaded PLLGA nanoparticles were prepared using an antisolvent diffusion method^{25, 27}. Briefly, 190 mg of PLLGA and 10 mg of RFX were dissolved in 4 mL of a mixed solution of acetone and ethanol (acetone:ethanol = 5:3). The solution was injected into 40 mL of purified water, 0.25, 0.50, 2.00, and 4.00% (w/v) PVA aqueous solution, and then the RFX-loaded PLLGA nanoparticles were immediately precipitated. The suspension was dialyzed for 24 h in a dialysis tube (C36-32-100, molecular weight cut-off: 14,000, EIDIA Co., Ltd., Tokyo, Japan) to remove unloaded-RFX. Purified water was used as the external liquid and was replaced after 2, 4, 6, and 8 h. After dialysis, RFX-loaded PLLGA nanoparticle suspensions were obtained. The mean volume diameters and size distributions of the nanoparticles were determined using a dynamic light scattering system (ELS-Z2, Otsuka Electronics Co., Ltd., Hirakata, Japan), which measures the scattered light that is generated when the laser light is irradiated onto particles that are in Brownian motion at 25°C²⁸.

2.3 Preparation of nanocomposite particles

The preparation of nanocomposite particles was performed similar to our previous study¹⁹. The nanoparticles were redispersed in purified water, and a physical mixture of arginine hydrochloride and leucine (arginine hydrochloride:leucine = 1:6) was added to an equal weight of precipitated nanoparticles. The suspensions were then spray-dried to prepare nanocomposite particles using a spray dryer (Mini Spray Dryer B-290, BÜCHI Corp., Flawil, Switzerland). Spray drying was performed under the following conditions: an outlet temperature of 37–40°C, an air volume of 22.5 m³/h, and a pump flow rate of 1.4 mL/min. After freezing at –30°C, the nanocomposite particles were lyophilized using a freeze dryer (FD-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) for 12 h. The size of the nanocomposite particles in the air was measured using a sizer (LD-SA-3500A, Nikkiso Co., Ltd., Tokyo, Japan). The mean volume diameters and the size distributions of PLLGA

nanoparticles redispersed from the nanocomposite particles were determined using a dynamic light scattering system (ELS-Z2) at 25°C. RFX content in the particles was measured using high-performance liquid chromatography (HPLC, SIL-20A prominence, SPD-20A prominence, LC-20AD prominence, CTO-10ASvp, DGU-20A3 prominence, Shimadzu Co., Kyoto, Japan) at 290 nm with an ODS column (STR ODS-M, size: 4.6 mm × 150 mm, Shinwa Chemical Industries Ltd., Kyoto, Japan). The mobile phase consisted of 52% water, 0.1% acetic acid, and 48% ethanol. Samples were filtered through a 0.45 µm pore membrane (Tokyo Roshi Kaisha, Ltd., Tokyo, Japan). The samples were dissolved in 10 mL of solution. HPLC measurements were performed at ambient temperature at a flow rate of 0.9 mL/min, and 20 µL of sample solution was applied. All HPLC measurements were performed under the same conditions²⁶.

2.4 *In vitro* release study of RFX from nanocomposite particles

To evaluate the effect of PVA on nanocomposite particles' drug release behavior, the release ratios of RFX from nanocomposite particles were determined. The cumulative release ratio was expressed as the ratio of RFX released from the particles divided by the amount of RFX initially contained in the nanocomposite particles²⁹. The particles were added to 500 mL of phosphate-buffered saline (PBS, pH 7.4) in a vessel to an RFX concentration of 1.0 µg/mL. Release tests were performed using a dissolution tester (NTR-6100A, Toyama Sangyo Co., Ltd., Osaka, Japan) at 100 rpm at 37°C. After 1, 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 min from the test initiation, 1.0 mL of each sample was collected. The cumulative release ratios of RFX from nanocomposite particles at each time point were calculated by measuring the amount of RFX using HPLC.

2.5 *In vitro* release study of RFX from redispersed RFX-loaded PLLGA nanoparticles

To evaluate the effect of PVA on drug release behavior, the release ratios of RFX from redispersed RFX-loaded PLLGA nanoparticles were determined. The cumulative release ratio was expressed as the ratio of RFX released from nanoparticles divided by the amount of RFX initially contained in the nanoparticles²⁹. Ten milligrams of nanocomposite particles were redispersed in 50 mL of PBS (pH 7.4) using a vortex mixer (Vortex-Genie 2, Electro Scientific Industries, Inc., Portland, OR). The suspension was diluted with PBS to an RFX concentration of 0.5 µg/mL. In total, 3 mL of the suspension was placed in a dialysis tube³⁰ and added to 97.0 mL of PBS (pH 7.4). The sample suspensions were shaken at 100 rpm and incubated at 37°C for 0.5, 1, 2, 4, 8, 12, or 24 h. The amount of RFX remaining in the dialysis membrane was quantified using HPLC. The amount of RFX released from the nanoparticles at each time was cal-

Fig. 2 Particle size distribution of nanocomposite particles containing RFX-loaded PLLGA nanoparticles ($n=3$).

culated by subtracting the measured residual amount from the initial amount²⁹⁾.

3 Results and Discussion

3.1 Characterization of nanocomposite particles and RFX-loaded PLLGA nanoparticles

The mean diameters of nanocomposite particles prepared using purified water, 0.25, 0.50, 2.00, or 4.00% (w/v) PVA aqueous solution were 2.86 ± 0.18 , 2.89 ± 0.10 , 4.33 ± 0.27 , 4.86 ± 0.27 , and 5.42 ± 1.12 μm , respectively. **Figure 2** shows the particle size distributions of the nanocomposite particles. The particle size increased as the concentration of PVA aqueous solution increased. The aerodynamic diameter, d_{aer} , of the particles is calculated using the following equation:

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

where d_{mass} , ρ , and F are the geometrical particle diameter, density of the particle, and shape function, respectively. The spherical particles are calculated with $F=1$ ^{31, 32)}. Therefore, an increase in the d_{mass} of nanocomposite particles due to an increase in the concentration of PVA aqueous solution may reduce their intrapulmonary delivery. RFX content in the particles prepared using purified water, 0.25, 0.50, 2.00, or 4.00% (w/v) PVA aqueous solution were 2.47 ± 0.02 , 2.44 ± 0.02 , 2.47 ± 0.01 , 2.45 ± 0.01 , and 2.46 ± 0.03 %, respectively. These results suggest that the concentration of PVA aqueous solution does not affect the RFX content of the particles. **Figure 3** shows the particle size distributions of RFX-loaded PLLGA nanoparticles. Their mean diameters and coefficients of variation are shown in **Table 1**. In the nanoparticles prepared using 0.25% (w/v) PVA aqueous solution, the redispersed nanoparticles had a smaller mean diameter and a higher

Fig. 3 Particle size distribution of RFX-loaded PLLGA nanoparticles ($n=3$). (a) immediately after preparation. (b) after redispersion from nanocomposite particles.

coefficient of variation than the nanoparticles immediately after preparation. PLGA nanoparticles have negative charges at neutral pH because of the ionization of the terminal carboxyl groups of PLGA³³⁾. This also applies to PLLGA nanoparticles, which contribute to improved dispersion stability of the particles in the solvent. In PLGA nanoparticles using PVA, it was reported that the negative charge derived from PLGA was shielded by the PVA covering the surface of the nanoparticles²⁵⁾. Therefore, we considered that the PVA-derived hydrated layer adsorbed on the surface contributes to the nanoparticles' dispersion stability. The instability of the nanoparticles prepared using 0.25% (w/v) PVA aqueous solution suggests that both the negative charge and the hydrated layer on their surfaces were insufficient. In the other nanoparticles, no significant changes were observed between those immediately after preparation and those after redispersion from nanocomposite particles. From these findings, we confirmed that various nanocomposite particles and PLLGA nanoparticles were successfully prepared.

Table 1 Mean volume diameters and coefficient of variation of PLLGA nanoparticles (mean $\pm$ S.D., $n=3$).

Poor solvent	Nanoparticles immediately after preparation		Nanoparticles after redispersion from nanocomposite particles	
	Mean volume diameter (nm)	Coefficient of variation	Mean volume diameter (nm)	Coefficient of variation
Purified water	160.5 $\pm$ 49.7	0.31	154.0 $\pm$ 48.3	0.31
0.25% (w/v) PVA	173.8 $\pm$ 44.7	0.26	149.5 $\pm$ 55.8	0.37
0.50% (w/v) PVA	150.4 $\pm$ 48.5	0.32	150.3 $\pm$ 47.1	0.31
2.00% (w/v) PVA	154.4 $\pm$ 51.4	0.33	160.6 $\pm$ 51.1	0.32
4.00% (w/v) PVA	159.5 $\pm$ 48.7	0.31	153.3 $\pm$ 45.0	0.29

Fig. 4 Cumulative release ratios of RFX from nanocomposite particles prepared using purified water, 0.25, 0.50, 2.00, and 4.00% (w/v) PVA aqueous solution (mean $\pm$ S.D., $n=3$, * $p<0.05$, Tukey's test).

3.2 *In vitro* release of RFX from nanocomposite particles

Figure 4 shows the cumulative release ratios of RFX from nanocomposite particles prepared using purified water, 0.25, 0.50, 2.00, and 4.00% (w/v) PVA aqueous solution at 37°C. The release ratios of the particles prepared using purified water, 0.25, 0.50, 2.00, and 4.00% (w/v) PVA aqueous solution after 10 min from the initiation of the tests were $36.2 \pm 8.4\%$, $85.9 \pm 4.9\%$, 93.1 ± 4.3 , 37.1 ± 3.1 , and $40.0 \pm 1.9\%$, respectively. In the particles prepared using 0.25% and 0.50% (w/v) PVA aqueous solution, the cumulative release ratios of RFX increased rapidly. This result suggests that a large amount of RFX was released from the nanoparticles, or the RFX-loaded nanoparticles were dispersed in the solvent due to efficient amino acid dissolution. In addition, a visual observation confirmed that these nanocomposite particles collapsed faster than the other particles. In the particles prepared using 2.00 and 4.00% (w/v) PVA aqueous solution, 40 min after the tests' initiation, the cumulative release ratios of RFX reached $85.0 \pm 9.5\%$ and $82.8 \pm 5.8\%$, respectively. It is challenging

to obtain molecularly dispersed PVA solutions in water³⁴⁾ PVA, which could not be adsorbed on the particle surface, physically mixed with the amino acids, and prevented their dissolution. The cumulative release ratio of RFX from the particles prepared using purified water did not reach 50% even after 1 h from the tests' initiation. We visually confirmed the presence of unbroken nanocomposite particles.

3.3 *In vitro* release of RFX from redispersed RFX-loaded PLLGA nanoparticles

The cumulative release ratios of RFX-loaded PLLGA nanoparticles prepared using purified water, 0.25, 0.50, 2.00, or 4.00% (w/v) PVA aqueous solution at 37°C are shown in Fig. 5. For all types of particles, the release ratios reached 97% 8 h after the initiation of the tests (Fig. 5a). Focusing on the early stages of drug release, we confirmed that after 0.5 and 1 h from the initiation of the tests, the release ratios of the nanoparticles prepared using 0.50 and 2.00% (w/v) PVA aqueous solution were significantly lower than those of the others (Fig. 5b). RFX is a hydrophobic drug and is almost insoluble in water³⁵⁾. These results indicate that the PVA-derived hydrated layer on their surfaces suppressed RFX release from the nanoparticles. However, the nanoparticles prepared using a 4.00% (w/v) PVA aqueous solution showed high release ratios similar to those prepared using purified water and a 0.25% (w/v) PVA aqueous solution. PVA has been reported to effectively increase the apparent solubility of poorly water-soluble drugs by functioning as a concentration-enhancing polymer²⁴⁾. Therefore, we considered that the presence of a large amount of PVA in the poor solvent increased the apparent solubility of RFX in water, and some RFX was transferred to water without being contained in the particles. The RFX transferred into the water was contained in the nanocomposite particles' amino acid portion and caused high drug release ratios in the early stages of drug release.

Fig. 5 Cumulative release ratios of RFX from PLLGA nanoparticles prepared using purified water, 0.25, 0.50, 2.00, and 4.00% (w/v) PVA aqueous solution after 0.5–24 h from the initiation of the tests (a) and 0.5–4 h from the initiation of the tests (b) (mean $\pm$ S.D., $n=3$, $*p<0.05$, $**p<0.01$, Tukey's test).

4 Conclusions

In this study, we controlled the drug release behavior of nanocomposite particles using PVA. The nanocomposite particles prepared using a 0.50% (w/v) PVA aqueous solution succeeded in the rapid release of nanoparticles from nanocomposite particles and suppressing the initial burst. These particles can be expected to redisperse into nanoparticles after reaching the lungs rapidly. This property is similar to that of nanocomposite particles prepared using conventional sugars. Therefore, these particles could be used to treat lung diseases and the stable storage of nanoparticles. Because the lungs have less water than the release test conditions used in this study, it may be necessary to develop nanocomposite particles that more easily release nanoparticles. Nanocomposite particles prepared using 0.25–2.00% (w/v) PVA aqueous solution should be studied in detail. The nanocomposite particles prepared using a 2.00% (w/v) PVA aqueous solution succeeded in the sustained release of nanoparticles from nanocomposite

particles and suppressing the initial burst. In addition to the release of the drug from the nanoparticles, these particles can be expected to be sustained-release in releasing the nanoparticles from the nanocomposite particles. The particles may reduce the frequency of drug administration in diseases that require long-term treatment, such as tuberculosis and lung cancer. This study conducted release tests on nanoparticles redispersed from nanocomposite particles to investigate the particles and drug behavior after reaching the lungs. In the future, to examine the effect of PVA on drug release behavior from nanoparticles in detail, it may be necessary to focus on nanoparticles immediately after preparation and nanoparticles from which surrounding drugs have been removed after redispersion.

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Conflict of Interest

The authors declare that they have no potential conflicts of interest.

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