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Inhalable nanocomposite particles using amino acids with improved drug content and humidity resistance

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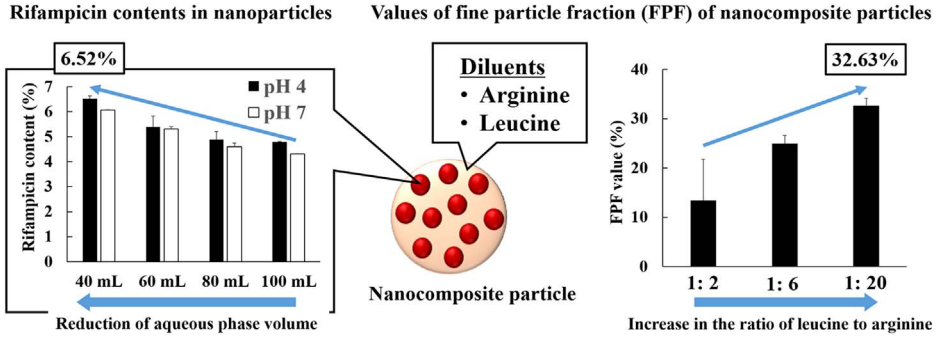
GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we prepared various rifampicin-loaded poly(DL-lactide-co-glycolide) nanoparticles by changing the volume and pH of the aqueous phase to improve the content of rifampicin in the nanoparticles. The rifampicin content in the nanoparticles prepared in the aqueous phase volume of 40 mL (pH 4) was 1.36 times higher than that in the nanoparticles prepared in the aqueous phase volume of 100 mL (pH 7). From the results of *in vitro* release tests in phosphate-buffered saline at 37 °C for 24 h, we found that by reducing the volume and pH of the aqueous phase, the cumulative release rate of rifampicin from nanoparticles was significantly decreased. Then, we prepared nanocomposite particles using arginine and leucine as diluents. The aerodynamic diameters of the nanocomposite particles were measured using a cascade impactor. We found that the nanocomposite particles prepared using a diluent with arginine to leucine ratio of 1: 20 had the highest fine particle fraction value (32.63 ± 1.49%) and the particles were suitable for pulmonary delivery of bioactive materials deep in the lungs.

1. Introduction

The lungs have a large surface area (43–102 m^2), thin absorption barrier and low enzymatic metabolic activity. In addition, drug delivery for local and systemic therapy *via* the lungs has many advantages over other delivery routes, due to slow mucociliary clearance of the alveoli and high permeability of the lung epithelia [1,2]. Recently, a number of pulmonary administration of drugs using fine particles has been studied [3]. Drug carriers, such as microparticles, nanoparticles, and liposomes, may enhance the systemic bioavailability of substances applied to the

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lungs by offering protection against enzymatic degradation, as well as by exhibiting controlled release properties [4–6]. Polymeric nanoparticles are promising carriers of biological agents to the lung due to advantages including biocompatibility, biodegradability, ease of surface modification, localization action, and reduced systemic toxicity. The most commonly used polymers for pulmonary administration include poly(lactic acid), poly(lactic-*co*-glycolic acid), poly(ε-caprolactone), alginate, chitosan, and gelatin base [7–10]. In the previous study, we have reported that local injection of anti-tubercle agents directly to the lungs using inhalable nanocomposite particles prepared using poly(DL-lactic-*co*-glycolic acid) (PLGA) nanoparticles containing rifampicin (RFP), a semi-synthetic bactericidal antibiotic drug for tuberculosis, and sugar [6,11,12]. The biocompatibility characteristics of PLGA have been reported [13]. Nanoparticles can overcome the mucus clearance [14], and they will be possible carriers of transporting drugs efficiently to the epithelia while avoiding unwanted mucociliary clearance. Also, it is reported that besides macrophages, other cells like cancer cells and epithelium cells are also able to take up nanoparticles [6,15–17]. This nanocomposite particle has an aerodynamic diameter suitable for inhalation. After reaching the alveoli, the nanocomposite particles are decomposed to nanoparticles, since the sugar moiety is soluble in the alveolar lining fluid [6]. However, RFP content in the primary nanoparticles was low (approximately 4%) [11], and when nanocomposite particles are prepared, the content further decreases. In addition, it was also a problem that the nanocomposite particles used trehalose dihydrate as the diluent. Trehalose dihydrate was suitable for lyophilizing the particles because it inhibits moisture absorption and has a tolerance against refrigeration [18]. In contrast, when preparing particles using spray drying, it was suggested that trehalose dihydrate often changes to anhydrous trehalose and hygroscopicity became high [19]. This would be an undesirable feature also *in vivo* experiments.

The aim of this study was to improve RFP content in primary nanoparticles and to investigate diluents for the preparation of nanocomposite particles with low hygroscopicity. We prepared PLGA nanoparticles with high drug content by changing the volume and pH of the aqueous phase for the preparation of O/W emulsion. Then, nanocomposite particles were prepared using the nanoparticles and amino acid. We selected arginine and leucine as diluents for the nanocomposite particles. Arginine was added as a dispersion stabilizer to redisperse the nanocomposite particles as the nanoparticles [20–22]. Leucine was added to reduce the aggregability of the particles prepared using spray drying [23]. To evaluate the usefulness of nanocomposite particles under high humidity and investigated effects of amino acid composition in the diluent on fine particle fraction (FPF) value, the FPF values under the condition of 90% relative humidity were measured.

2. Materials and methods

2.1. Materials

PLGA with a molecular weight of 10,000 and a DL-lactic acid/glycolic acid monomer composition of 75/25 (PLGA7510), polyvinyl alcohol (PVA, degree of polymerization: 500), trehalose dihydrate ($C_{12}H_{22}O_{11}\cdot 2H_2O$, purity ≥ 98%), citric acid monohydrate ($C_6H_8O_7\cdot H_2O$, purity ≥ 99.5%), acetonitrile (for HPLC, purity ≥ 99.8%), L(+)-arginine (purity ≥ 98%), and L-leucine (purity ≥ 99%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, 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. Preparation of RFP-loaded PLGA nanoparticles

RFP-loaded PLGA nanoparticles were prepared using the emulsion solvent evaporation method [24–27]. Briefly, 900 mg of PLGA7510 and 100 mg of RFP were dissolved in 20 mL of dichloromethane. The solution was added to 40, 60, 80, and 100 mL of 2.0% (w/v) of PVA aqueous solution (pH 7) or 2.0% (w/v) of PVA citric buffer solution (pH 4), and was emulsified using a probe sonicator (Digital Sonifier S-250D, Branson Ultrasonics Corp., Danbury, CT, USA) at 200 W of energy output. To prepare nanoparticles with a diameter of 200 nm, sonication was carried out under the conditions shown in Table 1 in an ice bath. The 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). Following centrifugation, the precipitated nanoparticles were rinsed with purified water in an ultrasonic bath sonicator to remove residual PVA. Centrifugation and rinsing were repeated for a total of three cycles.

Table 1
Sonication condition for preparation of RFP-loaded PLGA nanoparticles.

Volume of aqueous phase (mL)	Sonication time (s)
40	20
60	50
80	70
100	90

The mean volume diameters of RFP-loaded PLGA nanoparticles were measured using a particle size analyzer (ELSZ-2, Otsuka Electronics Co., Ltd., Osaka, Japan). Samples were dispersed in purified water and measured at 25 °C. 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-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). The mobile phase consisted of phosphate buffer solution (pH 2.6) and acetonitrile with a volume ratio of 2:3. The samples were dissolved in 10 mL of the solution. In addition, 30 mg of RFP was dissolved in 10 mL of the solution as a control. HPLC measurements were carried out at 40 °C (flow rate: 2 mL/min), and 50 μL of sample solution were applied. All HPLC measurements were carried out under the same conditions.

2.3. In vitro RFP release from RFP-loaded PLGA nanoparticles

To evaluate the influence of preparation conditions on drug release behavior, the release rates of RFP from the RFP-loaded PLGA nanoparticles were evaluated. Fifteen milligrams of RFP-loaded PLGA nanoparticles were redispersed in 5 mL of phosphate-buffered saline (pH 7.4). The sample solutions were shaken at 30 rpm at 37 °C. After 0.25, 0.5, 1, 2, 8, 24, and 48 h, the samples were centrifuged at 15,000 rpm for 10 min (Himac 80WX) [28]. Precipitates were collected and dissolved in 3 mL of the mobile phase. HPLC was used to determine the RFP concentration released from the nanoparticles.

2.4. Preparation of nanocomposite particles

The nanoparticles were redispersed in purified water, and arginine, leucine or their physical mixtures were added to an equal weight of precipitated nanoparticles. Then, the suspensions were spray-dried to prepare nanocomposite particles using a spray dryer (B-290, BUCHI Co., Ltd.) [6]. Spray drying was carried out under the following conditions, the inlet temperature of 37–40 °C, the air volume of 22.5 m^3/h, and pump flow rate of 1.2 mL/min.

The size of nanocomposite particles in the air was measured by using a sizer (LDSA-3500A, Nikkiso Co., Ltd., Tokyo, Japan). The mean volume diameters of RFP-loaded PLGA nanoparticles redispersed from the nanocomposite particles were measured using a particle size analyzer (ELSZ-2) at 25 °C. RFP contents in the nanocomposite particles were measured using HPLC. Surface properties of nanocomposite