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Research Paper

Preparation of Polymeric Submicron Particle-Containing Microparticles Using a 4-Fluid Nozzle Spray Drier

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Purpose. We studied a novel method for preparing polymeric submicron particle-containing microparticles using a 4-fluid nozzle spray drier.
Method. Ethylcellulose (EC) and poly(lactic-*co*-glycolic acid) (PLGA), either alone or in combination with polyethylenimine (PEI), were used as polymers to produce submicron particles, and mannitol (MAN) was used as a water-soluble carrier for the microparticles. The polymer and MAN solutions were supplied through different liquid passages of a 4-fluid nozzle and then dried to obtain MAN microparticles containing EC or PLGA submicron particles. The polymer/MAN ratio was controlled by changing the concentration of the polymer and MAN solutions. EC or PLGA microparticles were observed via scanning electron microscopy, and the size of microparticles was determined by image analysis. The particle size distribution of EC or PLGA submicron particles was measured with a super dynamic light scattering spectrophotometer.
Results. The method generated submicron-sized (<1 μm) particles of EC and PLGA. The mean diameters of EC and PLGA particles at a polymer/MAN ratio of 1:10 were 631 and 490 nm, respectively. The mean diameter of PLGA particles decreased as the PLGA/MAN ratio was reduced, reaching ~200 nm at a PLGA/MAN ratio of 1:100. The mean diameter of PLGA/PEI particles at PLGA/PEI/MAN ratios of 1:0.5:10 and 1:0.5:100 were 525 and 223 nm, respectively, and their zeta potentials were +50.8 and +58.2 mV, respectively. The size of EC submicron particles could be controlled by varying the spray conditions.
Conclusions. This study demonstrated that it is possible to prepare polymeric submicron particles dispersed in MAN microparticles in a single process using the 4-fluid nozzle spray drying method. Cationic PLGA particles with a diameter of ~200 nm could be prepared by adding PEI, suggesting the possibility of its use as a carrier for delivering DNA into cells. The precipitation of EC may occur by the mutual dispersion and mixing of solvents after collision of EC and MAN mists by antisolvent effect, thereby producing MAN microparticles containing EC submicron particles.

KEY WORDS: 4-fluid nozzle; microparticles; polymer; spray dry; submicron particles.

INTRODUCTION

Many recently developed drug delivery systems use various fine particles of drugs or carriers, depending on the physical properties of compounds and the dosage form and route of administration. Thus, particle design, in particular the design of micro-, submicron, or nanoparticles, is critical. Traditional methods for the production of particles include pulverization of large particles using a ball or jet mill, and solidification of emulsions by in-water drying methods. The first method is often used to improve the solubility of water-insoluble drugs. The dry grinding method may be limited to the preparation of particles down to several microns in diameter. Although the preparation of particles down to several hundreds of nanometers in diameter may be possible with the wet grinding method (1,2), it requires a large amount of cogrinding agents or surfactants, and the products are usually supplied as a suspension in which the solvent remains. These products are also susceptible to contamination by particles of ground metal. The second method can prepare particles from one to several hundreds of nanometers in diameter (3,4), but small changes in the preparation conditions may often induce a marked change in the physical properties of particles, and the preparation scale using this method is limited. In addition, the surface energy of the particle is extremely high for nanoscale particles. Therefore, it is difficult for them to exist in a solid state because of extremely high adhesion and cohesion forces, and, when the particles adhere, it is difficult to disperse them again, resulting in their loss of function as nanoparticles.

Therefore, a technology for preparing and collecting nanoparticles that avoids mutual cohesion is needed. However, this is not easy to achieve with traditional pharmaceutical technology. Supercritical CO_2 technologies have been

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recently employed to prepare microparticles (5–10). These technologies produce particles with high purity and low surface energy. This supercritical fluid technique is suitable for preparing particles of several microns, but is limited by the scale of preparation, the solubility of compounds in CO_2, and the lower size limit of the particles produced.

The 4-fluid nozzle spray drier has a unique nozzle containing two liquid and two gas passages, which allows two drugs to be dissolved in separate solvents, thereby overcoming problems of finding and using a common solvent. We have previously used this technique to prepare fine particles of pharmaceutical drugs for inhalation as a dry powder (11).

In this study, we attempted to prepare polymeric submicron particles dispersed within microparticles using the 4-fluid nozzle spray drying method. Ethylcellulose (EC) and poly(lactic-*co*-glycolic acid) (PLGA) were used as polymers for producing submicron particles. EC and PLGA have been used as polymers for controlled release (12–17) and targeting (18–21). Mannitol (MAN) was used as a water-soluble carrier for microparticles. We attempted to produce MAN composite microparticles containing EC or PLGA submicron particles. As recent investigations have examined the addition of a positive electric charge to carriers for the delivery of DNA into cells (22–24), we further attempted to prepare cationic PLGA submicron particles loaded with polyethyleneimine (PEI). Furthermore, we studied the control of the size of EC submicron particles and the mechanism by which they are produced.

MATERIAL AND METHODS

Materials

N-7 grade EC (EC-7; molecular weight = 58,000) and N-100 grade EC (EC-100; molecular weight = 230,000) were provided by Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). PLGA (lactic acid/glycolic acid ratio = 75:25; molecular weight = 14,400) and MAN were from Wako Pure Chemical Industries (Osaka, Japan). PEI (molecular weight = 50,000) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Methods

Preparation of Microparticles Containing Submicron Particles Using the 4-Fluid Nozzle Spray Drier

Spray-dried particles were prepared using a model MDL-050 4-fluid nozzle spray drier (Fujisaki Electric, Tokushima, Japan). Figure 1 depicts the 4-fluid nozzle that has two chisel-shaped nozzles through which compressed air passes and two additional nozzles through which sample solutions pass. Different liquids and gases can be individually supplied to each passage. In this study, compressed air was used as gas. The edge of the nozzle, which is a slit, functions as the liquid flow side at the time of operation. At the liquid flow sides, the sample solutions are withdrawn by high-speed compressed air in the acceleration zone. Air collides at the tip of the edge, and a powerful shock wave is generated at the collision focal spot. As a result, the drawn solutions are atomized into droplets. The droplets are then dried by heated air, and the dried particles are collected.

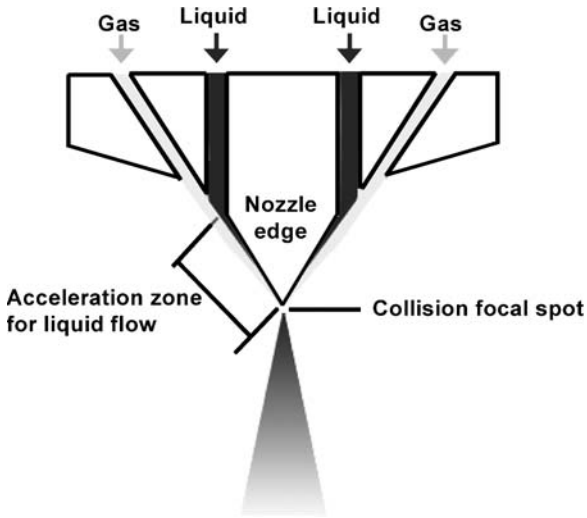

Fig. 1. Schematic diagram of the 4-fluid nozzle.

For the EC/MAN system, EC-7 was dissolved in ethyl acetate at 2.5% (w/v), and MAN was dissolved in water at 25% (w/v). This gave EC/MAN composition ratios of 1:10 (w/w). In the PLGA/MAN system, PLGA was dissolved in acetone/methanol (2:1) at 1.67% (w/v), and MAN was dissolved in water at either 1.67 or 16.7% (w/v). These yielded PLGA/MAN composition ratios of 1:1 and 1:10 (w/w). Alternatively, PLGA was dissolved in acetone/methanol (2:1) at 0.167% (w/v), and MAN was dissolved in water at 16.7% (w/v). This gave PLGA/MAN composition ratios of 1:100 (w/w). For the PLGA/PEI/MAN system, PEI was dissolved in water at 2 or 20% (w/v). The 20% (w/v) PEI aqueous solution was mixed with the 1.67% (w/v) PLGA solution to generate a PLGA/PEI/MAN ratio of 1:0.5:10, and the 2% (w/v) PEI aqueous solution was added to the 0.167% (w/v) PLGA solution to provide a PLGA/PEI/MAN ratio of 1:0.5:100. For studies on the control of EC submicron particles size, EC-7 or EC100 was dissolved in acetone/ethyl acetate (11:1) at 1.67% (w/v), and MAN was dissolved in water at 4.2, 8.3, or 16.7% (w/v). These gave EC/MAN ratios of 1:2.5, 1:5, and 1:10 (w/w). Also, EC-7 was dissolved in acetone/ethyl acetate (11:1) at 0.83 and 0.42% (w/v), and MAN was dissolved in water at 16.7% (w/v), giving EC/MAN composition ratios of 1:20 and 1:40 (w/w).

The polymer and MAN solutions were supplied through different liquid passages of the 4-fluid nozzle. Spray drying was typically performed under the following conditions: inlet temperature, 60°C; outlet temperature, 35–39°C; supply rate for polymer and MAN solutions, 2.5 mL/min; atomizing air volume, 20 L/min; and spray air pressure, 8 kgf/cm^2.

Scanning Electron Microscopy (SEM)

Particles were observed under an S-2250N scanning electron microscope (Hitachi, Tokyo, Japan). The EC/MAN or PLGA/MAN microparticles were coated with 25-nm-thick gold using a model SC-701 quick carbon coater (Sanyu Electronics, Tokyo, Japan). For observation of the EC and PLGA submicron particles, the EC/MAN or PLGA/MAN microparticles were placed in ultrapure water to dissolve the MAN. The EC or PLGA suspensions were filtered through a 0.22-μm cellulose acetate membrane filter (Advantech Japan,

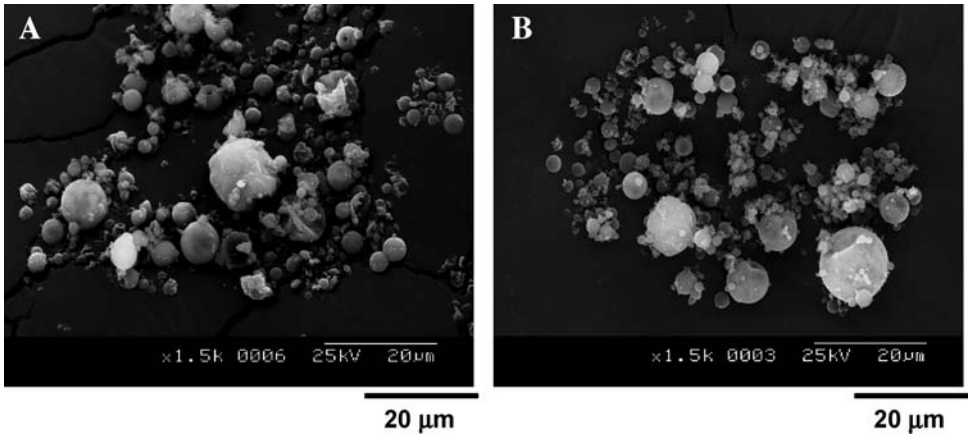

Fig. 2. SEM photographs of (A) EC/MAN and (B) PLGA/MAN microparticles. EC/MAN and PLGA/MAN ratios = 1:10.

Tokyo, Japan). Filtration was repeated several times to remove MAN. The membrane filter was then removed and thoroughly dried. The membrane filter was coated with 25-nm-thick gold and the EC or PLGA particles remaining on the membrane filter were observed.

Measurement of the Mean Particle Diameter (D_{50}) of EC/MAN and PLGA/MAN Microparticles

The particle diameters (the horizontal Feret diameter) of the EC/MAN and PLGA/MAN microparticles were determined by image analysis from approximately 500 particles using WinROOF image analysis software (Mitani, Fukui, Japan). D_{50} was defined as the median diameter from the cumulative curve of the number-basis particle size distribution.

Measurement of Particle Diameter of EC and PLGA Submicron Particles

EC/MAN and PLGA/MAN microparticles were placed in ultrapure water to dissolve the MAN. The particle size distribution of EC or PLGA in the suspension was directly measured by using a model DLS-7000 super dynamic light scattering spectrophotometer (Otsuka Electronics, Osaka, Japan) without filtration of the EC and PLGA suspensions after MAN is dissolved. EC 0.42% (w/v) acetone/ethyl acetate (11:1) solution and MAN 16.7% aqueous solution, which correspond to a EC/MAN composition ratio of 1:40 (w/w), were mixed in a test tube and the particle size distribution of EC in the suspension was also measured. D_{50} was defined as the median diameter of the cumulative curve of the scattering intensity-basis particle size distribution. Measurement was performed under the following conditions: EC or PLGA concentration, 0.05 mg/mL; Ar laser output, 5 A; angle of laser, 90°.

Measurement of the Zeta Potential of PLGA Submicron Particles

The zeta potential of PLGA submicron particles and PLGA/PEI submicron particles was measured using a model 501 Laser Zee zeta potential meter (Pen Kem, Inc., Bedford Hills, NY, USA).

RESULTS AND DISCUSSION

Observation of EC/MAN and PLGA/MAN Microparticles

SEM images of microparticles of EC/MAN (1:10) and PLGA/MAN (1:10) are shown in Fig. 2A and B, respectively. Both types of particles were nearly spherical. The mean diameters of the EC/MAN and PLGA/MAN particles were 3.1 and 2.5 µm, respectively.

Particle Size Distribution and SEM Imaging of EC and PLGA Particles Dispersed in MAN Microparticles

Polymer suspensions were obtained by placing EC/MAN and PLGA/MAN microparticles in ultrapure water to

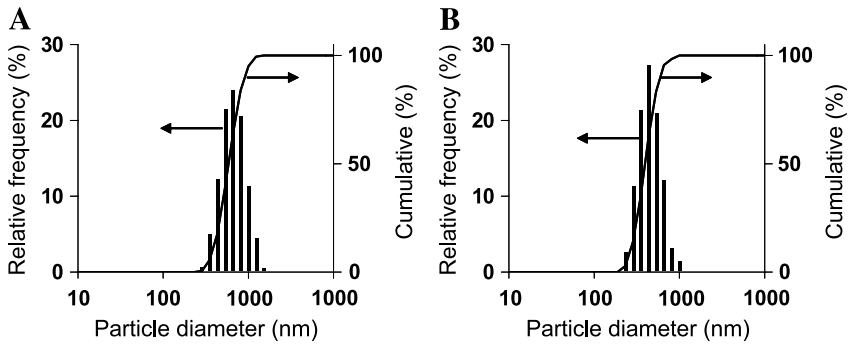

Fig. 3. Particle size distributions of (A) EC and (B) PLGA particles.

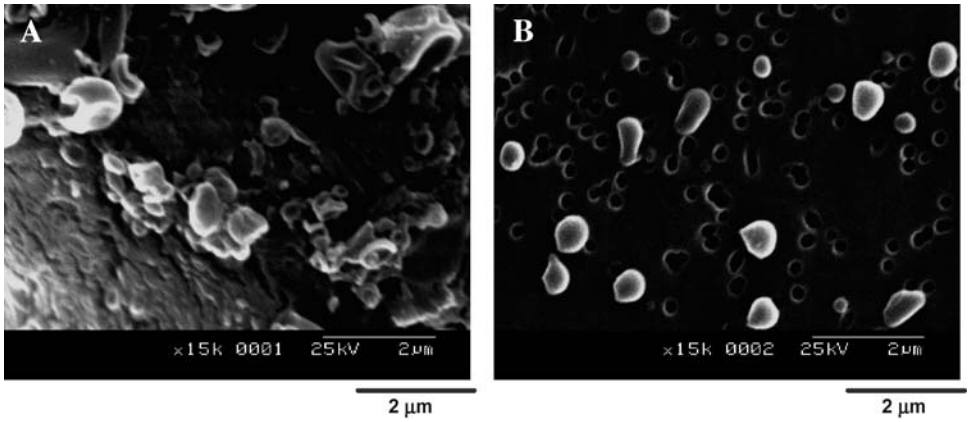

Fig. 4. SEM photographs of (A) EC and (B) PLGA particles dispersed in MAN microparticles after the removal of MAN.

dissolve MAN without filtration. The size distributions of EC and PLGA particles are shown in Fig. 3A and B, respectively. In both cases, the particles were submicron-sized (i.e., diameters < 1 μm). The D_{50} of EC and PLGA were 631 and 490 nm, respectively. The SEM images of EC and PLGA particles dispersed in MAN after removal of MAN are shown in Fig. 4A and B, respectively. The EC particles were somewhat irregular in shape, whereas the PLGA particles were nearly spherical. Although it is not completely clear, it seems that EC has a high spinnability.

These results show that it is feasible to use the 4-fluid nozzle spray drying method to prepare EC and PLGA submicron particles dispersed in MAN microparticles. These polymeric submicron particle-containing microspheres can be directly used for oral and mucosal administration of drugs, or they can be dissolved just before injection. Furthermore, because the microspheres contain very fine microparticles and the size of microparticles can be reduced by changing the spray conditions (11), they could be used for delivery of drugs via dry powder inhalation. Also, the presence of MAN in the microparticles maintains the physical stability and prevents the aggregation of the nanoparticles.

Preparation of Cationic PLGA Submicron Particles Dispersed in MAN Microparticles

We next examined the effect of adding a positive charge to the surface of the PLGA by adding PEI. Microparticles of PLGA/PEI/MAN with a 1:0.5:10 ratio were prepared by spray drying as described for the PLGA/MAN preparation. The PLGA/PEI/MAN microparticles were placed into ultrapure water to dissolve MAN. The particle size distribution of the resulting PLGA/PEI particles was wider than that of PLGA particles (Fig. 3). In addition, the D_{50} of PLGA/PEI particles (525 nm) was slightly increased compared to PLGA particles, and the zeta potentials of PLGA and PLGA/PEI particles were −24.8 and +50.8 mV, respectively (data not shown). These results show that it is possible to prepare cationic submicron particles by adding PEI along with the anionic PLGA using the 4-fluid nozzle spray drier. These cationic PLGA submicron particles may be able to adsorb DNA onto their surfaces and may therefore be useful as a carrier for the delivery of DNA into cells.

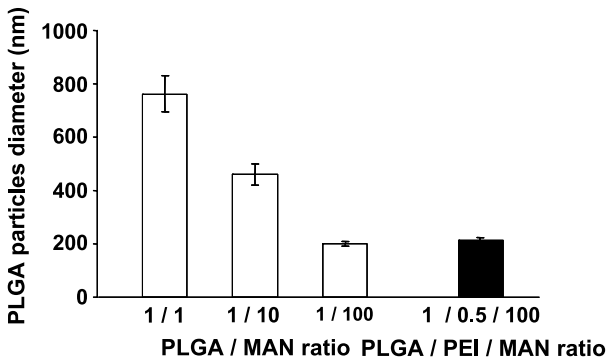

Fig. 5. D_{50}s of PLGA and PLGA/PEI particles dispersed in PLGA/MAN and PLGA/PEI/MAN microparticles after dissolution of MAN at various composition ratios (mean ± SD, $n = 3$).

Effect of PLGA/MAN Ratio on the Size of PLGA Submicron Particles

We next investigated the effect of changing the PLGA/MAN ratio on the size of submicron particles. The PLGA/MAN ratio of the submicron particles was controlled by changing the concentration of PLGA or MAN solutions. We prepared composite microparticles at PLGA/MAN ratios of 1:1, 1:10, and 1:100 and at PLGA/PEI/MAN ratios of 1:0.5:100. Figure 5 shows the mean diameter of PLGA and PLGA/PEI particles after MAN is dissolved with water. The diameter of PLGA particles decreased as the PLGA ratio was lowered. The D_{50} of PLGA particles at PLGA/MAN ratios of 1:1, 1:10, and 1:100 was 763, 490, and 194 nm, respectively, and that of PLGA/PEI particles was 223 nm. The zeta potential of PLGA/PEI particles was +58.2 mV. Thus, it was possible to prepare cationic PLGA submicron particles with a diameter of ~200 nm.

Control of Submicron Particle Size and Mechanism of Production

MAN microparticles containing EC submicron particles were prepared at various EC/MAN ratios and with different sizes of EC. For experiments examining the effect of different EC/MAN ratios EC-7 was used, and for experiments examining the effect of different molecular weights of

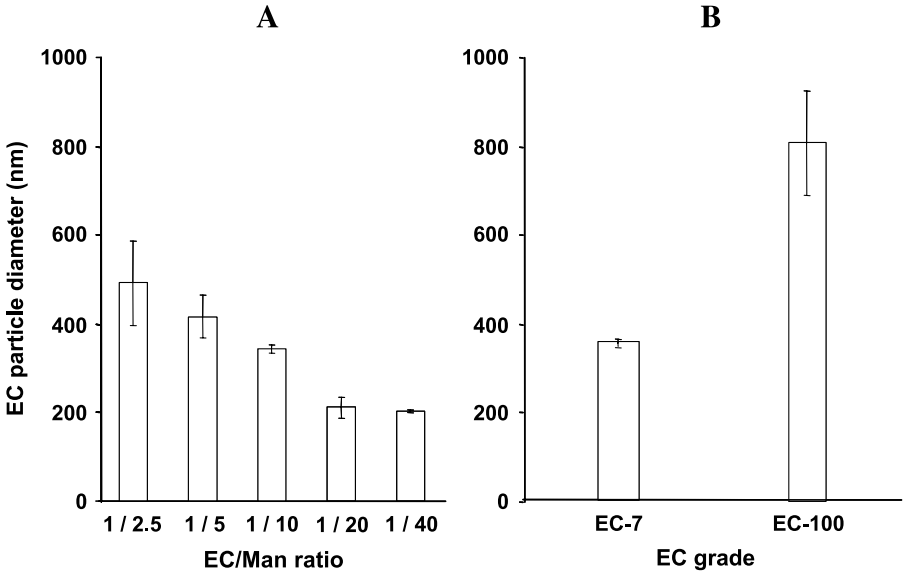

Fig. 6. Effect of (A) EC/MAN ratio and (B) EC molecular weight on the D_{50} of EC particles (mean ± SD, n = 3). Spray drying was performed under the following conditions: inlet temperature, 60°C; outlet temperature, 28–32°C; supply rate for polymer and MAN solutions, 5 mL/min; atomizing air volume, 30 L/min, spray air pressure, 8 kgf/cm^2.

EC the EC/MAN ratio was fixed at 1:10. The size of EC/MAN microparticles at various EC/MAN ratios and molecular weights of EC was almost the same (1.6 *vs.* 2.4 μm). The effects of EC/MAN ratio and EC molecular weight on the mean diameter of EC particles are shown in Fig. 6A and B, respectively. The diameter of EC submicron particles decreased as the EC/MAN ratio decreased. The diameter of EC submicron particles decreased to ~200 nm at an EC/MAN ratio of 1:20. Furthermore, the diameter of EC submicron particles was smaller with the lower molecular weight of EC.

We next examined the effect of preparing MAN microparticles containing EC submicron particles at various inlet temperatures and atomizing air volumes. In both cases, EC-7 was used and the EC/MAN ratio was fixed at 1:10. The size of EC/MAN microparticles at various inlet temperatures remained essentially unchanged at ~1.8 μm and the size of EC/MAN microparticles at atomizing air volumes of 10 and 30 L/min was 1.8 and 2.4 μm, respectively. The effects of inlet temperature and atomizing air volume on the mean diameter of EC particles are shown in Fig. 7A and B, respectively. The diameter of EC submicron particles tended to decrease as the inlet temperature was lowered, and it became significantly smaller when the temperature was dropped to 40°C. In addition, results show that lower atomizing air volumes produce EC submicron particles having a smaller diameter. Preparation of EC submicron particles at the lowest EC/

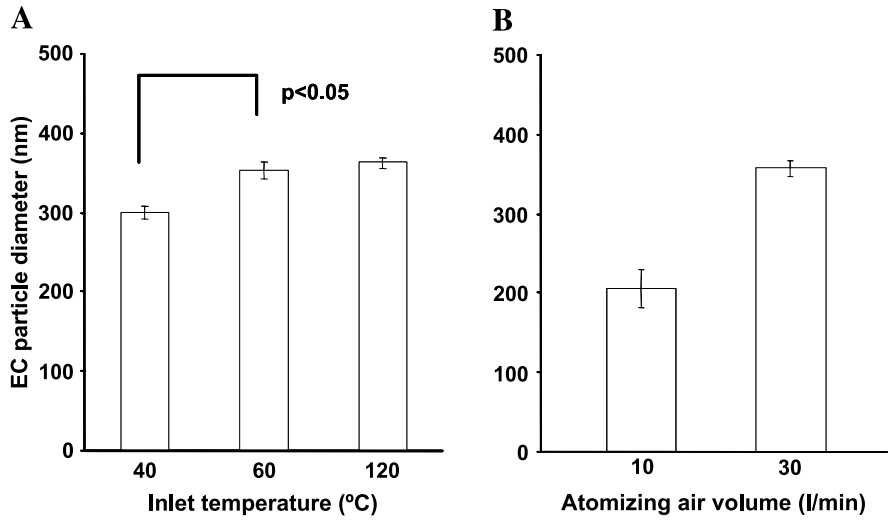

Fig. 7. Effect of (A) inlet temperature and (B) spray air volume on D_{50} of EC particles (mean ± SD, n = 3). Atomizing air volume was fixed at 30 L/min, and for experiments at various atomizing air volumes, inlet temperature was fixed at 60°C.

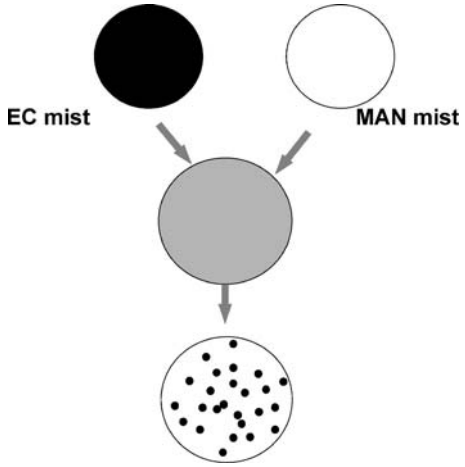

Fig. 8. Illustration of a conceivable mechanism for producing EC submicron particles in MAN microparticles.

MAN ratio (1:40), molecular weight of EC (EC-7), inlet temperature (40°C), and atomizing air volume (10 L/min) used in this study generated EC particles with a mean diameter of 160 nm.

The diameter of EC particles dispersed in EC/MAN microparticles decreased as EC/MAN ratio, molecular weight of EC, inlet temperature, and atomizing air volume were decreased. In other words, the diameter of EC submicron particles decreased upon reduction of the concentration of EC compared to MAN, the viscosity of EC solution, the drying rate of spray mist, and the air collision force at the tip of the 4-fluid nozzle. When the air collision force was smaller, the size of spray mist became larger, causing a lower drying rate. Figure 8 shows a conceivable mechanism of the production of EC submicron particles in MAN microparticles. Mutual dispersion and mixing of the solvents occur immediately after the EC and MAN mists collide. This results in the precipitation of EC by antisolvent effect, producing the EC submicron particle-containing MAN microparticles. A lower concentration and viscosity of the EC solution may generate finer nuclei of EC, thus producing smaller EC submicron particles. A lower drying rate of the spray mists may cause a significant mutual dispersion and mixing of the solvents in the mists so that a number of fine nuclei of EC can be formed. PLGA nanoparticles may be formed by the same mechanism. The EC solution and MAN solution, which correspond to the EC/MAN composition ratio of 1:40 (w/w), were mixed and the particle size distribution of EC in the suspension was also measured. The diameter of EC submicron particles was 124 ± 18 nm (n = 3), which is smaller than that of EC prepared by using the 4-fluid spray drier. This result may support the consideration for the mechanism of the production of EC submicron particles by antisolvent precipitation of EC in the mist.

CONCLUSIONS

This study demonstrated that it is possible to produce polymeric submicron particles dispersed in MAN microparticles in a single step using a 4-fluid nozzle spray drying method. In addition, cationic PLGA submicron particles were prepared by adding PEI, suggesting that they may be useful as a carrier for the delivery of DNA into cells. The size of EC submicron particles could be controlled by varying the spraying conditions, and particles as small as 160 nm in diameter could be prepared. The precipitation of EC, which produces EC submicron particle-containing MAN microparticles, may occur via the mutual dispersion and mixing of solvents after the collision of EC and MAN mists. This technology may be developed to produce microparticles for oral and pulmonary administration or injection of water-insoluble drugs.

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