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ORIGINAL ARTICLE

Preparation of griseofulvin nanoparticle suspension by high-pressure homogenization and preservation of the suspension with saccharides and sugar alcohols

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Abstract

Aim: We have attempted to micronize drug particles with a particle size of less than 100 nm and maintain the particle size of their suspension to improve the solubility and bioavailability of poorly water-soluble drugs. Furthermore, the method of freeze-drying nanoparticles was applied to maintain particulate nature of nanoparticles containing various saccharides and sugar alcohols for a long time. *Method*: Griseofulvin (GF)–lipid nanoparticle suspension is prepared using GF and a lipid by high-pressure homogenization. The particle size of the obtained GF–lipid nanoparticle suspension is maintained constant by freeze-drying. *Result*: The mean particle size of GF–lipid nanoparticles prepared by high-pressure homogenization is approximately 60 nm. The mean particle size remains less than 100 nm for 1 month. The GF–lipid nanoparticle suspension containing xylitol, trehalose, or sucrose is freeze-dried to maintain the particulate nature. The mean particle size of the rehydrated suspension is lower than that of the rehydrated suspension containing erythritol or lactose. In particular, it is new knowledge to have found that an aggregation is minimized by adding xylitol which is sugar alcohol. The minimum concentration of xylitol, trehalose, and sucrose required to maintain a constant particle size by rehydration is 3%, 3%, and 5% (w/v), respectively.

Key words: *Freeze-drying; high-pressure homogenization; nanoparticle suspension; particle size; rehydration; saccharides; sugar alcohols*

Introduction

Improvement in the bioavailability of poorly water-soluble drugs is expected to enhance their dissolution in gastrointestinal fluids. To increase their solubility in water surfactants are used[1–9] and they are co-ground with water-soluble polymers[10–14]. We have focused on the process of micronization of drug particles. Although mainly polymers are used in the preparation of nanoparticles, the toxicity of polymers and the use of surfactants pose problems. Although it is simple to micronize liposomes or polymer emulsions containing water-soluble drugs, it is very difficult to micronize poorly water-soluble drugs for a particle size less than 100 nm. Thus far, for example, griseofulvin (GF), which is a practically insoluble drug, was micronized to a mean particle size of 200 nm by high-pressure homogenization[15]. The aims of this study are to (1) prepare less toxic nanoparticles and large batches of nanoparticles with a particle size of less than 100 nm by simple and useful methods and (2) maintain the particulate nature of the nanoparticle suspension containing various saccharides and sugar alcohols by freeze-drying.

In this study, GF is used as a model[16]. GF is an antibiotic and antifungal drug administered predominantly in oral doses. It has a very low solubility (15 μg/mL at 37°C)[17]; hence, the absorption of this drug is very low in the gastrointestinal tract (GIT) at the time of fasting. Therefore, it

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is important to pulverize drug particles to the order of nanometers in order to increase its solubility[18–21].

Micronization of drug particles is expected to improve drug solubility and thereby aid the delivery of drug particles into the systemic circulation through Peyer's patches in the GIT. The application of a wet process along with a combination of mechanical methods can reduce the particle size to the order of several hundreds of nanometers, whereas the application of a dry process can reduce the particle size to the order of several micrometers[22,23].

Kraml et al.[24] have reported that the serum levels produced by a dose of 0.5 g of micronized GF are identical to those produced by a dose of 1.0 g of non-micronized GF; hence, it is feasible to improve the low serum levels, produced due to the low absorption of drugs, by the administration of micronized GF. Additionally, the bioavailability of GF is reportedly improved as the specific surface area of the particles increased[16]. Therefore, absorption through GIT and bioavailability are expected to be enhanced by reducing the size of GF particles. In a study of absorption, different particle sizes of polystyrene microspheres (50–3000 nm) are administered to rats daily for 10 days to investigate uptake across the gastrointestinal mucosa[25]. The fraction absorbed is 5% for particles of less than 1000 nm, 15% for particles of less than 500 nm, and 26% for particles of less than 100 nm, demonstrating that the fraction absorbed depended on particle size.

The method of freeze-drying nanoparticles was applied to maintain particulate nature of nanoparticles prepared by high-pressure homogenization for a long time[26]. Moreover, the recoverability of the rehydrated nanoparticle suspension, which was freeze-dried along with various saccharides and sugar alcohols, was investigated in detail to preserve the particulate nature of suspension for a long time[27]. Hitherto, saccharides and sugar alcohols (e.g., monosaccharide and disaccharide) were mainly used as a lyo-protectant during the process of freeze-drying. However, little has been reported about using sugar alcohols (e.g., xylitol and erythritol) for freeze-drying. Therefore, we have investigated the relationship between the particulate nature and sugar alcohol.

Materials and methods

Materials

Hydrogenated soybean phosphatidylcholine (COATSOME® NC-21; HSPC) was purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). Dicetyl phosphate (DCP; SIGMA®) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). GriseoFulvin (JPXIV) was provided by Nippon Fine Chemical Co., Ltd. (Tokyo, Japan). Ethanol, lactose, xylitol, and sucrose (reagent grade) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Trehalose and erythritol were provided by Fuji Nihon Seito Co. (Tokyo, Japan). A membrane filter (pore size: 0.45 μm) was purchased from Toyo Roshi Kaisha Ltd. (Tokyo, Japan). The reagents were used as they were obtained. Purified water treated by ion exchange was used.

Preparation of GF–lipid nanoparticle suspension

In a 80°C water bath, 20 mg of GF and 1000 mg of a lipid (HSPC/DC: 5:1 molar ratio) were dissolved with 2 mL of ethanol, and ethanol was evaporated. The mixture was then dispersed in 200 mL of purified water and premixed using a TK homomixer (10,000 rpm; Tokushu Kika Kogoyo Co., Tokyo, Japan) for 15 minutes. This premixed suspension was applied in a high-pressure homogenizer (max pressure: 9.5 kg/cm^2; Nanomizer, X form chamber; Tokushu Kika Kogyo Co.) and processed for 10, 20, 30, or 40 cycles of homogenization (number of cycles: pass number).

Nanoparticle size measurement

The mean particle size of the nanoparticle suspension prepared by high-pressure homogenization for different numbers of rotations was measured at room temperature using an electrophoretic light scattering photometer (ELS; ELS-8000, Otsuka Electronics Co., Ltd.) at a fixed angle of 90°. The particle size was analyzed on the basis of the weight distribution of the nanoparticle suspension. The nanoparticle suspensions were analyzed without dilution.

Freeze-drying and rehydration methods

Freeze-drying method: 2 mL of the GF–lipid nanoparticle suspension was collected in separate vials, and 100 mg of sucrose, xylitol, trehalose, erythritol, or lactose was added to the vials. Each vial was vortexed, and the suspensions were frozen at −35°C and left standing for 24 hours. The frozen samples were freeze-dried in a glass chamber for 24 hours using a vacuum pump and vapor condenser (−90°C, 1.0×10^{-3} torr; Neocool; Yamato Scientific Co., Ltd., Tokyo, Japan).

Rehydration method: 2 mL of purified water was added into the vials and shaken by hand to rehydrate the freeze-dried samples. The mean particle size of the rehydrated GF–lipid nanoparticle suspensions was determined by using the ELS.

Results and discussion

Pulverization of drug particles by high-pressure homogenization and characteristics of nanoparticles

We have attempted to prepare a drug–lipid nanoparticle suspension with a particle size of less than 100 nm

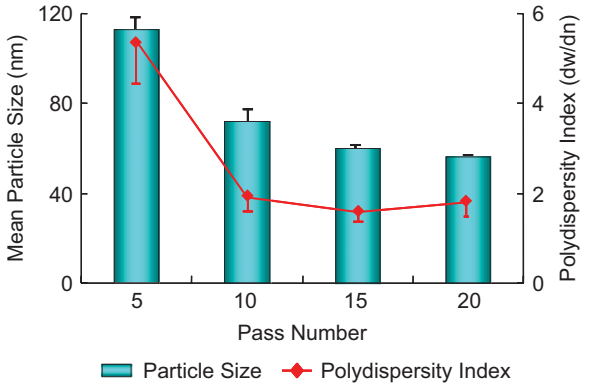

Figure 1. Influence of pass number on mean particle size and polydispersity index of GF–lipid nanoparticle suspension prepared by high-pressure homogenization. Columns indicate the relationship between pass number and mean particle size. Diamonds indicate the relationship between pass number and polydispersity index. Each bar represents the mean ± SD of three measurements.

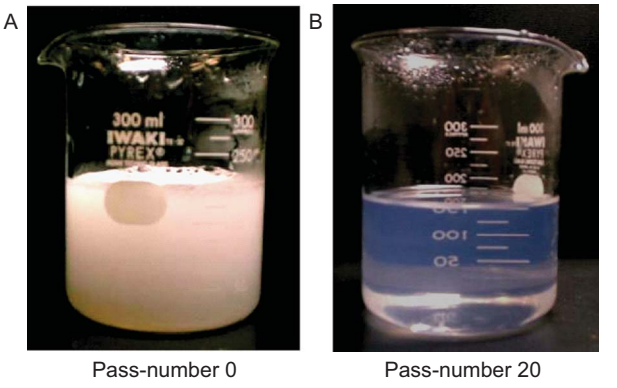

Figure 2. Photographs of GF–lipid nanoparticle suspension: (A) before and (B) after high-pressure homogenization.

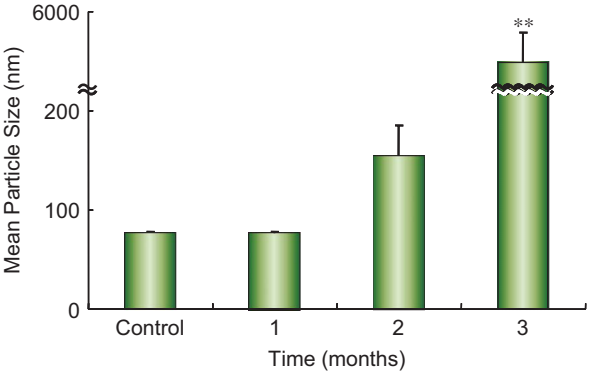

Figure 3. Particle size consistency of GF–lipid nanoparticle suspension at 4°C. Columns indicate the relationship between time and the mean particle size. Control: the mean particle size immediately after high-pressure homogenization. Student's *t*-test is used for statistical analysis. Significant deviation from the mean particle size of the control group is indicated by $^{**}P<0.01$. Each bar represents the mean ± SD of three measurements.

because the solubility of drugs is closely associated with the surface area of drug particles[28–31]. Figure 1 shows the influence of pass number during high-pressure homogenization on the mean particle size and polydispersity index. The mean particle size of the suspension decreases with an increase in the pass number[32–35], and the mean particle size is approximately 60 nm when the pass number is 20. The polydispersity index also decreases with an increase in the pass number[36], and the polydispersity index is approximately 1 when the pass number is 10, suggesting that larger particles are pulverized preferentially. When the particle size distribution approaches a monodisperse distribution, the particles are completely pulverized while the monodispersity of the particle is maintained[37]. It is not shown in a figure. We attempted to prepare nanoparticles with a GF/lipid ratio: 1:25 (40:1000 mg), 1:33.3 (30:1000 mg), 1:40 (25:1000 mg), and 1:66.7 (15:1000 mg). Nanoparticles with a particle size less than 100 nm and with only the ratio of 1:66.7 (GF/lipid) could be prepared. However, others show the particle size of nanoparticles more than 1000 nm. The ratio of GF and lipid definitely affect their particle size. Therefore, the ratio of 1:20 (GF/lipid) is the best selection to prepare nanoparticles.

Figure 2 shows the photographs of the premixed suspension before high-pressure homogenization (A) and the GF–lipid nanoparticle suspension (B). The suspension shown in Figure 2B was very transparent, indicating that the GF particles have been micronized completely. Adkins et al. have reported that an appearance of nanoparticles with a particle size ranging from 50 to 200 nm shows transparent liquid[38,39]. The GF particles appear to be micronized adequately from its appearance.

Figure 3 shows the stability of dispersion of the GF–lipid nanoparticle suspension. The mean particle size is approximately 70 and 130 nm after 1 and 2 months, respectively. The GF–lipid nanoparticles aggregated and thereby the suspension became opaque after 3 months. Meanwhile, the suspension placed at room temperature for 2 weeks turned opaque (data not shown). Morale et al. have reported that an aggregation behavior of nanoparticles took place by an individual random dispersal described from a Brownian motion[40–43]. Further, Brownian motion has strong relationship with temperature[44–48]. The aggregation of particles appeared to be suppressed in the former case because the motion of the GF–lipid nanoparticles when the suspension was placed in a cool and dark place is slower than that of when the suspension is placed at room temperature.

The application of freeze-drying methods was investigated for the long-term stabilization of the GF–lipid nanoparticles[49–53]. This method was applied to preserve

the particulate nature. Furthermore, we also investigated the effects of the addition of saccharides and sugar alcohols on the preservation of the particle size of the GF–lipid nanoparticle suspension. Thus far, it has been reported that saccharides (sucrose[54–56] and trehalose[57–59]) could maintain their particle size in the case of freeze-drying. However, little has been reported that sugar alcohols prevented an aggregation of nanoparticles in the case of freeze-drying. Because sugar alcohol is low in calories compared with saccharides, this study is useful to a hyperglycemic patient.

Rehydration of freeze-dried nanoparticle suspension and effect of saccharides and sugar alcohols on particulate nature

The mean particle size of the rehydrated GF–lipid nanoparticle suspension containing 5% (w/v) saccharides and sugar alcohols. The mean particle size is approximately 6000–10,000 nm when erythritol or lactose is added to the suspension. Meanwhile, the mean particle size is approximately 200 nm when xylitol, trehalose, or sucrose is added to the suspension, suggesting that these three types of saccharides and sugar alcohol can prevent the aggregation of nanoparticles. In particular, it is new knowledge to have found that an aggregation is prevented by adding xylitol which is sugar alcohol. There are many reports of freeze-drying nanoparticles containing phosphatidic acid which is negatively charged lipid[60–62]. However, there are no or few reports of freeze-drying nanoparticles containing DCP. It may not be much universal to use DCP during freeze-drying. Increase of particle size may be related to affinity of DCP and HSPC.

Crowe et al. have reported that saccharides interact with the nanoparticles by directly forming H-bonds with their surface, producing the steric effect, thereby preventing the aggregation of particles[63]. A similar phenomenon has taken place in this study. The interaction between the hydrogen groups of nanoparticles and xylitol, trehalose, or sucrose appears to be sufficient because the GF–lipid nanoparticles have been rehydrated completely. In addition, the interaction between the hydrogen groups of nanoparticles and erythritol, or lactose, does not appear to exist completely. Although xylitol and erythritol are sugar alcohols, it is difficult to explain that the rehydrated particle size containing xylitol is different from the rehydrated particle size containing erythritol.

Figure 4A and 4B shows the photographs of freeze-dried and rehydrated GF–lipid nanoparticle suspension containing trehalose and lactose, respectively, as a representative example. The sucrose and xylitol-additive nanoparticles redisperse to form suspensions similar to that of 4A, whereas erythritol-additive nanoparticle

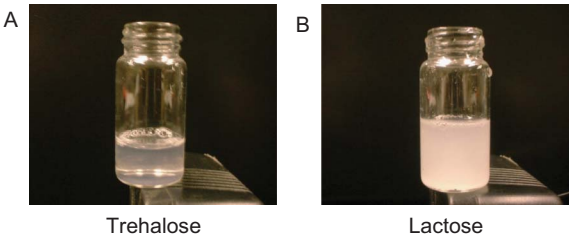

Figure 4. Photographs of rehydrated GF–lipid nanoparticle suspension containing saccharide: (A) GF–lipid nanoparticle suspension with trehalose after rehydration and (B) GF–lipid nanoparticle suspension with lactose after rehydration.

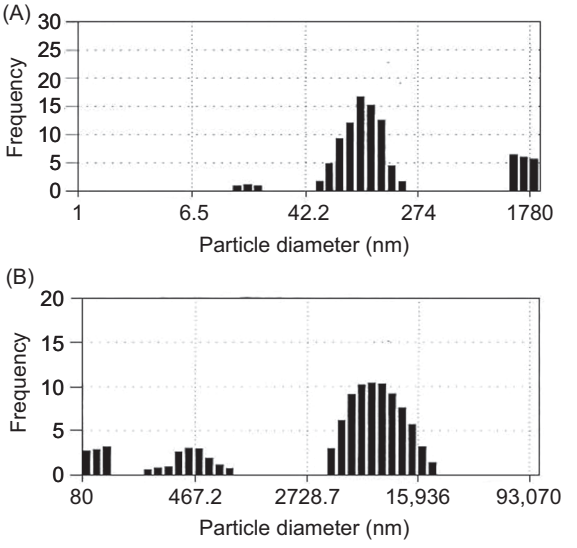

Figure 5. Particle size distribution charts of rehydrated GF–lipid nanoparticle suspension containing (A) trehalose and (B) lactose. GF–lipid nanoparticle suspension containing various saccharides and sugar alcohols is rehydrated in distilled water immediately after freeze-dried.

suspension seems to be similar to that of 4B. The latter suspension is opaque in comparison with the former.

Figure 5A and 5B show the distribution charts of the scattering intensity to elucidate the particle size distribution of rehydrated GF–lipid nanoparticle suspensions containing trehalose and lactose, respectively. Both distribution charts show three peaks. The main distribution appears for particle sizes of approximately 200 nm in the case of trehalose. However, the largest distribution is observed for the particle sizes exceeding 500 nm in the case of lactose. These distribution charts show that the particle size distribution for the GF–lipid nanoparticle suspension containing trehalose is clearly smaller than that of the GF–lipid nanoparticle suspension containing lactose.

The particle size distribution chart shown in Figure 5A proved that two particle size distributions exist for particle size of approximately 200 and 1800 nm, respectively. Thus, a fraction of the distribution around 1800 nm appeared to

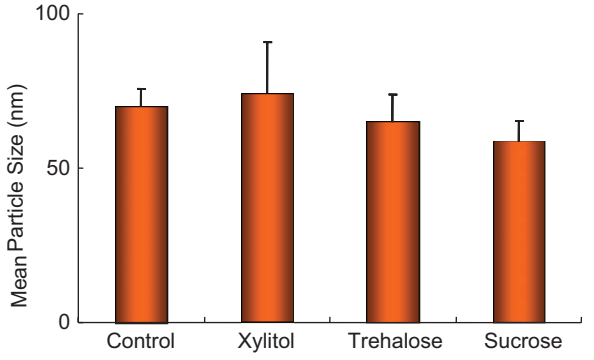

Figure 6. Mean particle size of filtrated GF-lipid nanoparticle suspension. Columns indicate the mean particle size of rehydrated GF–lipid nanoparticle suspension, which has been filtrated using the membrane filter (pore size: 0.45 μm). Control: the mean particle size of GF–lipid nanoparticle suspension before freeze-drying. Each bar represents the mean ± SD of three measurements.

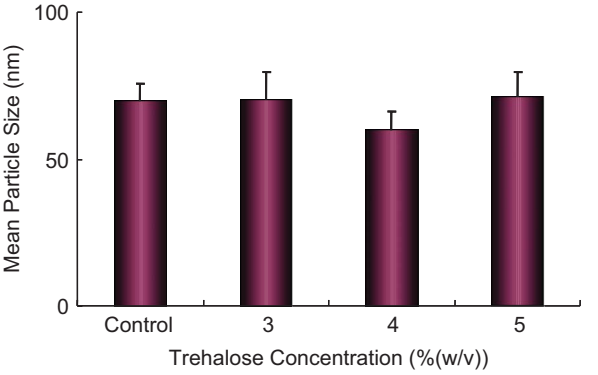

Figure 7. Mean particle size of GF–lipid nanoparticle suspension. Columns indicate the relationship between concentration of trehalose and mean particle size of the GF–lipid nanoparticle suspension, which has been filtered using the membrane filter (pore size: 0.45 μm). Control: the mean particle size of GF–lipid nanoparticle suspension before freeze-drying. Each bar represents the mean ± SD of three measurements.

raise a value of the mean particle size. From this view, we determine the mean particle size of the particles that do not exhibit aggregation (i.e., particles with particle size less than 0.45 μm). Figure 6 shows the mean particle size of the suspension containing xylitol, trehalose, or sucrose; the suspension has been freeze-dried and rehydrated and subsequently filtered using membrane filter (pore size: 0.45 μm). The mean particle size of the GF–lipid nanoparticle suspension containing xylitol, trehalose, or sucrose is less than 100 nm and is similar to that of GF–lipid nanoparticle suspension before freeze-drying. However, it is impossible to determine the particle size of the rehydrated suspension containing erythritol or lactose in a part without aggregation because of the clogging of the filter. From the above-mentioned findings, it is confirmed that xylitol, trehalose, and sucrose can restore the particle size in rehydrated GF–lipid nanoparticle suspensions, which has been freeze-dried.

Cui et al.[64] have reported that sucrose (1–5%, w/v) is required for successful lyophilization of the nanoparticles. Therefore, we have tried to find a minimum concentration of saccharides and sugar alcohol. Figure 7 shows the mean particle size of the rehydrated GF–lipid nanoparticle suspension containing various concentrations of trehalose. Although the particle size of the rehydrated suspension is constant at a concentration of 3–5% (w/v), it is impossible to determine the particle size of the GF–lipid nanoparticle suspension for concentrations less than 2% (w/v) because of the noticeable particle aggregation. In conclusion, it has been revealed that a small amount of saccharide or sugar alcohol is essential to maintain the particle size during the process of freeze-drying. The minimum concentration of trehalose, sucrose, and xylitol required is 3%, 3%, and 5% (w/v), respectively (data not shown). Because H-bonds between nanoparticles and xylitol are weaker than the other saccharides, the minimum concentration of xylitol is higher. Furthermore, Li et al.[65] have also proposed that sugars have the ability to form a rigid sugar glass or vitrified network structure in which liposomes can be embedded. Trehalose 3% (w/v), sucrose 3% (w/v), and xylitol 5% (w/v) appear to be sufficient concentration to embed GF–lipid nanoparticles.

Conclusion

The results are summarized as follows:

1. The mean particle size of the GF–lipid nanoparticle suspension prepared by high-pressure homogenization was 60 nm when the pass number was 20. The GF particles could be micronized adequately under 100 nm by using high-pressure homogenizer.
2. The appearance of the GF–lipid particles remained stable for 1 month in a cool and dark place. However, the GF–lipid nanoparticle suspension showed aggregation within 2 weeks at room temperature. The aggregation of the nanoparticles was suppressed in the former case because the particles motion in the cool and dark space was slower than that of the suspension placed at room temperature.
3. The mean particle size of the freeze-dried and rehydrated GF–lipid nanoparticle suspension containing xylitol, trehalose, or sucrose was similar to that of the suspension before freeze-drying. Xylitol, trehalose, and sucrose have prevented the aggregation of the nanoparticles. It is new knowledge to have found that xylitol could prevent an aggregation of the nanoparticles. The minimum concentration of trehalose, sucrose, and xylitol required is 3%, 3%, and 5% (w/v), respectively.

Declaration of interest: The authors report no conflicts of interest.

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