Spray freeze-drying (SFD) technique using four-fluid nozzle (4N), which is a novel particle design technique previously developed by authors, has been further developed to expand its application in pharmaceutical industry. The organic solvent was utilized as a spray solvent to dissolve the poorly soluble drug instead of conventional aqueous solution. Acetonitrile solution of the drug and aqueous solution of the polymeric carrier were separately and simultaneously atomized through 4N, and collided each other at the tip of nozzle edge. The spray mists were immediately frozen in the liquid nitrogen to form a suspension. Then, the iced droplets were freeze-dried to prepare the composite particles of the drug and carrier according to our proprietary method developed before. The resultant composite particles with phenytoin prepared by using acetonitrile (4N-SFD-MeCN system) were deeply characterized compared to those using aqueous solution (4N-SFD-aqua system) from morphological and physicochemical perspectives. The characteristic porous structure was observed in 4N-SFD-MeCN particles as well as 4N-SFD-aqua particles. However, it was found that the size and quantity of pore in 4N-SFD-MeCN particles were smaller than those of 4N-SFD-aqua particles. As a result, the former particles had 2- to 3-times smaller specific surface area than the latter particles independent of the type of carrier loaded. The slight difference of release profiles from the particles prepared between both systems was discussed from the microscopically structural viewpoint. In addition, ciclosporin was applied to organic solvent SFD system because this drug was poorly water soluble and cannot be applied to conventional aqueous SFD system. The release profiles from SFD particles were dramatically improved compared to the bulk material, suggesting that the new SFD technique using organic solvent has potential to develop the novel solubilized formulation for poorly water-soluble active pharmaceutical ingredients (APIs).

Key words spray freeze-drying; liquid nitrogen; four-fluid nozzle; porous microparticle; acetonitrile
pH range was not enough to apply to 4N-SFD-aqua process. The effect of the composition ratio between ciclosporin and water-soluble carrier on in vitro dissolution behavior was extensively investigated.

**Experimental**

**Materials** Phenytion and ciclosporin, as an active pharmaceutical drug, were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Methacrylic acid copolymer (Eudragit L100) and hydroxypolymethacrylate-lulose (TC-5, type R) were provided by Evonik Degussa Japan Co., Ltd. (Tokyo, Japan) and Shin-etsu Co., Ltd. (Tokyo, Japan), respectively. Phenytion, ciclosporin, Eudragit L100 and hydroxypolymethacrylate-lulose were abbreviated to Phe, Cic, Eud-L and HPMC in this report, respectively. The polymeric additives to make the composite particles with drug were comprehensively called “carrier” in this paper. Actonitritile, shortly described as MeCN in this paper, was purchased from Wako Pure Chemical Co., Ltd. All other chemicals and solvents were of analytical reagent grade, and deionized-distilled water was used throughout the study.

**Spray Freeze-Drying (SFD) Procedure** The manufacturing apparatus and operational procedure for SFD technique have been reported in our previous paper. The four-fluid nozzle (4N, Fujiwaki Electric Co., Ltd., Tokushima, Japan) was used in this study. The freeze-dryer (PER-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) connected to the trapping unit (UT-2000, Tokyo Rikakikai) was used to remove the iced water, iced MeCN and residual liquid nitrogen from the resultant composite particles.

**Spray Freeze-Drying (SFD) Preparation** The spray-dryer with four-fluid nozzle (MDL-050B, Fujiwaki Electric Co., Ltd., Tokushima, Japan) was used in this research. The freeze-dryer (PER-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) connected to the trapping unit (UT-2000, Tokyo Rikakikai) was used to remove the iced water, iced MeCN and residual liquid nitrogen from the resultant composite particles.

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order to clarify the physicochemical and pharmaceutical differentiation of both products obtained by 4N-SFD using organic and aqueous solvent systems, the 4N-SFD-MeCN and 4N-SFD-aqua composite particles were prepared in same condition with fixing the ratio of phenytoin against the carrier to 1 : 5. Either Eud-L or HPMC as a carrier was formulated in the particles. The morphological appearances of each composite particle were observed by SEM as shown in Fig. 1. It was found that 4N-SFD-MeCN particles (B-1, 2, 3) have porous and spherical structure, which is same characteristic morphology exhibited in 4N-SFD-aqua particles (A-1, 2, 3) as also reported in previous paper.20,21) No difference in particle size was visually confirmed and Phe : Eud-L particles from both systems had 2—25 μm in diameter as shown in low-magnified photographs (A-3, B-3). However, the size and quantity of pores observed in the 4N-SFD-MeCN particles (B) are apparently smaller than those of 4N-SFD-aqua particles (A). Such SEM observation was highly consistent with the results of specific surface area as shown in Table 2. That is, the 4N-SFD-MeCN particles had 2- to 3-times smaller specific surface area than the 4N-SFD-aqua particles even if either carrier is used. It is assumed that the difference of specific surface area is caused by volume change when liquid is frozen. Namely, the volume of organic solvents including MeCN decreases when solidified by cooling below freezing point, whereas the volume of water increase when it changes to ice. The pores are assumed to be formed as a remained void, i.e. a vestige, after ice crystals disappeared. Therefore, the 4N-SFD-aqua droplets, which were swollen at freezing, could result in the particles with larger porous structure. The reason why the Phe : HPMC = 1 : 5 particles had smaller specific surface area than Phe : Eud-L = 1 : 5 particles in the 4N-SFD-MeCN system is not clear, but the precipitation process might be different in the both carriers because of poor solubility of HPMC in MeCN, whereas good of Eud-L. Anyway, it should be emphasized that the specific surface area of SFD particles had 50 times or over larger than the bulk material of phenytoin even in case of 4N-SFD-MeCN particles. With respect to the polymeric carrier formulated in the particles, Eud-L-based particles (A-2, B-2) were observed to have higher spherical shape with rigid framework compared to HPMC-based particles (A-1, B-1). The satellite particles less than 1 μm in diameter were also observed in SEM photographs of 4N-SFD-MeCN products, which are assumed to be generated by splattering at collision between droplets.

The cumulative particle size distribution (PSD) curves of Phe : Eud-L = 1 : 5 particles prepared by both solvent systems are shown in Fig. 2. It was found that there was clear difference in PSD between both systems. The mean particle sizes

![Fig. 1. Scanning Electron Microphotographs of 4N-SFD Composite Particles Prepared by A) 4N-SFD-Aqua System and B) 4N-SFD-MeCN System](image)

![Fig. 2. Cumulative Particle Size Distribution Profiles of Phe : Eud-L = 1 : 5 Composite Particles Prepared by 4N-SFD-Aqua and 4N-SFD-MeCN Systems](image)

<table>
<thead>
<tr>
<th>Sample/solvent system</th>
<th>Specific surface area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4N-SFD-aqua</td>
</tr>
<tr>
<td>Phe : HPMC = 1 : 5</td>
<td>129</td>
</tr>
<tr>
<td>Phe : Eud-L = 1 : 5</td>
<td>126</td>
</tr>
<tr>
<td>Bulk material of phenytoin</td>
<td>0.77</td>
</tr>
</tbody>
</table>
that are median diameters obtained from the each cumulative curve are 27.7 and 17.5 μm for 4N-SFD-aqua and 4N-SFD-MeCN methods, respectively. PSD measured by laser diffraction scattering method were a little larger than the apparent size observed by SEM photographs (Fig. 1). The particles might be somewhat aggregated during measurement dispersed by dry air because of their cohesive property. Anyway, the smaller surface tension of MeCN (19.1 dyn/cm) than that of water (72.7 dyn/cm) might result in finely splattered mists of MeCN solution as explained by Raghavan et al.29 As a result, the smaller particles were prepared from MeCN solution, which sizes are directly related to those of spray mists because the mists were freeze-dried while keeping their sizes and shapes. In addition, the difference in particle size would be also attributed to the volume change of MeCN and water when frozen as mentioned above. As a result, the particles obtained from 4N-SFD-MeCN was assumed to be little smaller than those from 4N-SFD-aqua. These results revealed that the current SFD technique using the four fluid-nozzle could successfully produce the composite particles with single-micron to 25 μm in size even if MeCN was used as a spray solvent. The spherical SFD composite particles were also prepared with high yield (>90%) in any systems because there is no product loss such as leakage from filter or adhesion to parts of instrument basically.

The crystalline property of phenytoin loaded in the SFD composite particles prepared by both solvent systems was examined by X-ray powder diffraction (XRPD) and DCS. The XRPD patterns and DSC curves of the SFD particles (Phe : carrier = 1 : 5) are shown side by side in Fig. 3 together with phenytoin original bulk. These results indicated that the drug was assumed to be amorphous in the particles prepared by both systems (4N-SFD-aqua, 4N-SFD-MeCN) since both diffraction peaks and endothermic peak derived from the crystal of the drug were completely disappeared. Based on the results, it was concluded that the SFD particles with 5-times loading of carrier to the drug form a solid dispersion in which phenytoin is dispersed completely in the polymer matrix with no crystal.

The release property from the SFD composite particles was examined in the media adjusted at pH 1.2 and 6.8 to simulate the gastrointestinal environments. The release profiles of Phe : HPMC = 1 : 5 composite particles prepared by both solvent systems indicated that the release of the drug from SFD particles were considerably improved in comparison to the phenytoin bulk powder in both media as shown in Fig. 4. Such rapid release behavior of the particles is due in part to the hydrophilic and water-soluble characteristics of HPMC independent of pH. In addition, the enhanced surface area resulting from porous structure and amorphous nature of the drug in the particles also might accelerate the dissolution. Comparing the profiles between two 4N-SFD particles (aqua and MeCN systems), the release of 4N-SFD-MeCN particles was a bit faster than that of 4N-SFD-aqua particles, although the former had smaller specific surface area than the latter. The lack of consistency is assumed to be caused by partial formation of HPMC-rich domain with hydrophilic property inside the particles. 4N-SFD-MeCN particles were prepared by mixing the MeCN solution of phenytoin and aqueous HPMC solution. Immediate freezing just after mixing of such heterogeneous solvents might result in partial phase separation in the droplets and finally partial segregation of polymeric phase and drug phase in the particles. It is assumed that these segregations were not generated in crystalline level, but in more tiny microscopic level because the phenytoin was proved to be dispersed as amorphous state in the particles as shown in Fig. 3. As a result, the hydrophilic HPMC-rich phase actively promoted the penetration of dissolution medium into 4N-SFD-MeCN particles.

On the other hand, the release profiles of Phe : Eud-L = 1 : 5 composite particles were illustrated in Fig. 5. The SFD particles with Eud-L showed delayed release compared to the phenytoin bulk material in the acidic medium. Contrary to HPMC case mentioned above (Fig. 4), the release of 4N-SFD-MeCN particles was not so delayed as seen in 4N-SFD-aqua particles. The weak sustained-release behavior of 4N-
SFD-MeCN particles would be also caused by partial segregation in molecular level. The spotty dispersed phenytoin-rich domain, which constructs the acid-philic phase scattered in the acid-phobic Eud-L matrix on the surface of the particles, could disturb the shutout of dissolution media penetrating into the particles, resulting in inhibition of anti-acidic property. In contrast, the release profiles of the drug from both SFD particles were considerably improved in the medium at pH 6.8, attaining almost 100% release within 30—40 min. These enteric release behaviors are considered to be attributed to pH-dependent property of Eud-L dissolved in the acid-phobic Eud-L matrix on the surface of the particles, resulting in inhibition of anti-acidic property. In contrast, the release profiles of the drug from both SFD particles were considerably improved in the medium at pH 6.8, attaining almost 100% release within 30—40 min. These enteric release behaviors are considered to be attributed to pH-dependent property of Eud-L dissolved in the acid-phobic Eud-L matrix on the surface of the particles, resulting in inhibition of anti-acidic property.

Loading of Ciclosporin in SFD-MeCN Composite Particles

Ciclosporin is currently used as an immunosuppressant for the treatment of a number of autoimmune diseases and reported to have a variety of biological activities. Ciclosporin is currently used as an immunosuppressant for the treatment of a number of autoimmune diseases and reported to have a variety of biological activities.

The formulation development of ciclosporin was sometimes troublesome because of its poor solubility. The application to the current SFD technique is also limited since this drug could not be dissolved in any aqueous solution at wide pH range. In fact, although the authors tried to apply ciclosporin to the 4N-SFD-aqua system we could not realize the preparation because aqueous solvent to dissolve was not found at the concentration level for the standard preparation. Therefore, the SFD particles containing ciclosporin was prepared in the 4N-SFD-MeCN system by using acetonitrile as its good solvent. HPMC is coloaded in SFD particles to improve the dissolution property of this poorly water-soluble drug. The particles having 1:4, 1:5, 1:7 weight ratio of ciclosporin to HPMC, abbreviated as Cic: HPMC=1:4, 1:5, 1:7, respectively, were prepared to evaluate the influence of composition.

The SEM photographs (Fig. 6) revealed that the particles had spherical shape and characteristic nanosized-porous structure in every drug ratio as also seen in phenytoin-loaded particles. It was also found that the particles with increasing HPMC ratio became finer texture. The SEM observation has good agreement with the values of specific surface area tabulated in Table 3. The particles with higher HPMC content had larger specific surface area. The polymeric additives seem to be attributed to forming fine network structure resulting from fiber-like precipitation at freezing. Anyway, the specific surface area increased 10-times larger than that of original bulk of ciclosporin even in the smallest values (24.6 m²/g) of Cic: HPMC=1:4. The SEM observation at low-powered magnification exhibited no particle size difference among the particles with 1:4, 1:5, 1:7 contents. The results of XRPD and DCS indicated that ciclosporin was perfectly dispersed as amorphous status in polymeric matrix of the particles with every composition though the actual scanning curves were not shown in this report.

The release profiles of Cic: HPMC=1:4, 1:5, 1:7 particles were plotted in Fig. 7 in the media adjusted at pH 1.2 and 6.8. The release of ciclosporin bulk material was found to be quite slow due to the practically insoluble property in spite of amorphous bulk used, attaining to less than 10% even at 300 min. In contrast, the release of ciclosporin from every SFD particles was considerably improved to that of bulk material in the both media, reaching up to almost 100% at 180 min. Comparing the release behaviors among the particles with different ratio of Cic: HPMC, the particles with higher HPMC content showed the faster release rate. Especially, the particles with Cis: HPMC=1:7 showed almost perfect dissolution within initial 60 min. Such aggressive dissolution is considered to be caused by increased surface area and higher content of water soluble carrier of the particles.

Table 3. Specific Surface Area of Ciclosporin Bulk Material and SFD Composite Particles with Ciclosporin Prepared by 4N-SFD-MeCN System

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composite particles with Cic : HPMC</th>
<th>Cic bulk material</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:4</td>
<td>1:5</td>
</tr>
<tr>
<td>Specific surface area (m²/g)</td>
<td>24.6</td>
<td>29.7</td>
</tr>
</tbody>
</table>

Fig. 7. Release Profiles of Ciclosporin from Cic/HPMC Composite Particles Prepared by 4N-SFD-MeCN System (Left: pH 1.2, Right: pH 6.8)


Conclusion

In the present research, the SFD technique using the four-fluid nozzle, previously developed by authors, was further improved by adopting acetonitrile as a spray solvent to expand the application of the poorly water soluble drugs. The composite particles including the polymeric carrier, which plays a role of dissolution modifier, were successfully prepared without changing the standard condition of preparation.
established before. That is to say, the aqueous solvent was only replaced with acetonitrile, having good solubilized power and relatively high freezing point, to dissolve the drug and other condition was not changed. This replacement allows drug and dissolution modifier to be dissolved in separate solvents such as organic and aqueous solvents, overcoming problems with finding and using a common solvent. It was found that the particles obtained by acetonitrile system (4N-SFD-MeCN) had characteristic internal structure with infinite pores, a bit smaller particle size and 2- to 3-times smaller specific surface area compared to the 4N-SFD-aqua particles. The drug release patterns were dependent on the solubility of the carrier formulated, that is to say, rapid release property in case of HPMC and enteric release property in case of Eud-L as a carrier. Those results indicated that the 4N-SFD-MeCN technique is quite useful to develop the novel formulation for solubilization of the poorly water soluble or practically insoluble drugs, such as ciclosporin. Especially, the porous SFD particles having 1—10 μm diameter and low particle density could have high potential for pulmonary delivery. Our research to apply the SFD particles to dry powder inhaler is progressing and will be reported in our following papers.

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References