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Design of porous microparticles with single-micron size by novel spray freeze-drying technique using four-fluid nozzle

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ABSTRACT

Spray freeze-drying (SFD) process, which is a novel particle design technique previously developed by authors, has been improved by using four-fluid nozzle (4N) instead of conventional two-fluid nozzle (2N) to expand its application in pharmaceutical industry. Aqueous spray solutions of the drug and the polymeric carrier were separately supplied into 4N, and atomized while colliding with each other at the tip of nozzle. The droplets of mixed solutions were directly immersed into liquid nitrogen and immediately frozen to form a suspension. Then, the iced droplets were lyophilized by freeze-dryer to prepare the composite particles of the drug and carrier. This process has been used in the present study to modify and enhance the dissolution profiles of poorly water-soluble drug, phenytoin. Water-soluble and enteric polymeric carriers in pharmaceutical use were used as a dissolution modifier. The SFD composite particles prepared by using 4N were fully characterized compared to those using 2N from morphological and physicochemical perspectives. It was found that the particles have fine porous structure producing vast specific surface area. Further, phenytoin was completely dispersed as amorphous state in the polymeric matrix with higher carrier ratio than phenytoin:carrier = 1:3. The dissolution of phenytoin from the water-soluble carrier-based particles was greatly enhanced because of large effective surface area and disappearance of crystalline. On the other hand, the release profiles from enteric carrier-based particles showed the typical enteric patterns, that is, delayed in acidic medium and accelerated in neutral pH. The results demonstrated that SFD technique using 4N has potential to develop the novel solubilized formulation for poorly water-soluble APIs.

1. Introduction

During the discovery and preclinical/clinical development stages of pharmaceutical products, many pharmacological and toxicological researchers often face the problem of poor oral absorption caused by poor aqueous solubility of the active pharmaceutical ingredients (APIs). They sometimes push back to medicinal chemists to retry chemical modification of lead compounds to seek more promising compound, so-called "candidate". Whereas, chemical as well as biological researchers frequently tend to put their hope on formulation technologies enhancing the dissolution of poorly water-soluble API because it is quite difficult to introduce the hydrophilic moiety in the candidate remaining its high biological potency. In fact, more than 40% of the candidates in the development pipelines are reported to be categorized as poorly soluble (Prentis et al., 1988; Lipinski, 2000). Formulation technologies that have been commonly used to achieve this task include mechanical milling (Merisko-Liversidge et al., 2003; Bahl et al., 2006; Pongpeerapat et al., 2008), hot melt extrusion (Gupta et al., 2002), coprecipitation (Sinswat et al., 2005), complex formation with water-soluble excipients (Wang, 2000; Hussein et al., 2007), spray-drying (Chen et al., 2004; Janssens et al., 2009), and freeze-drying (Ahmed et al., 2007), and so on. Among technologies above, spray-drying has been widely applied in pharmaceutical as well as food industries because it is suitable for industrial production. Furthermore, it is advantageous to poorly water-soluble drugs to produce the spherical and size-controlled particles and to improve dissolution property simultaneously. However, this technique is not always appropriate for thermolabile or oxidizable APIs such as biological drugs because its process requires heating and air-blowing.

Spray freezing into liquid (SFL) is a novel particle engineering technology developed by Rogers et al. (2002a,b, 2003), in which a feed solution of an API and a pharmaceutical excipient, so-called carrier, is atomized beneath the surface of a cryogenic liquid nitrogen. The particles are prepared by freeze-drying of the frozen droplets. Authors also have developed spray freeze-drying (SFD) technique, which is similar to SFL, to increase universality of unique lyophilization technique in SFL (Kondo et al., 2009). SFD technique is combined with the conventional spray-dryer with freeze-dryer,

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both equipments are available in market. In this process a spray solution of an API and carrier is atomized over the surface of liquid nitrogen using the nozzle part of spray-dryer, and then the frozen suspension in cryogenic liquid is transferred into freeze-dryer to obtain the dried particulate powder. This cryogenic technique has several advantages compared to the conventional spray-drying and/or freeze-drying as follows: (1) process with no heat and air supply is applicable to labile APIs; (2) atomization and following freezing procedures produce spherical particles with controllable size; (3) rapid freezing of fine droplets minimizes the crystallization and phase separation of drug; (4) finely dispersion into iced particles promotes accelerated and uniform drying; (5) removal of ice in small droplets makes the porous structure. These advantages sound very attractive to develop the composite particles of poorly water-soluble APIs from the industrial perspective.

In this research, SFD technique was further improved by adopting four-fluid nozzle (4N) to expand its application in pharmaceutical industry. Authors have already developed 4N spray-drying technique to design functional composite particles with sustained and enhanced release behaviors (Chen et al., 2006b, 2008). In conventional SFL and SFD methods using two-fluid nozzle (2N), an API and a pharmaceutical carrier have to be dissolved together in a common solvent because 2N has only one feed line for liquid. Finding a common solvent would sometimes lead to restricted application of these techniques. 4N having two liquid passages allow an API and a carrier to be dissolved in separate solvents, overcoming this problem (Ozeki et al., 2005). For example, poorly water-soluble API dissolves in organic solvent and dissolution-accelerated carrier dissolves in water, which is likely common case, increasing the chance of combination between the API and the dissolution modifier. In the current report, we investigated the manufacturability of 4N-SFD technique using only aqueous solution prior to combination sprays of aqueous and organic solutions. To attain this object, the SFD particles were prepared in the following three systems: (1) 2N-spray system for a common aqueous API/carrier solution; (2) 4N-spray system for a common aqueous API/excipient solution; (3) 4N-spray system for separate aqueous API solution and aqueous carrier solution. Phenytoin was used as poorly water-soluble drug and two types of carrier were used as a dissolution modifier. The composite particles obtained by three SFD methods were characterized in the viewpoints of physicochemical and dissolution properties.

2. Materials and methods

2.1. Chemicals

Phenytoin was purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Methacrylic acid copolymer (Eudragit L100) and hydroxylpropylmethylcellulose (TC-5, type R) were provided Evonik Degussa Japan Co., Ltd. (Tokyo, Japan) and Shin-Etsu Co., Ltd. (Tokyo, Japan), respectively. Phenytoin, Eudragit L100 and hydroxylpropylmethylcellulose were abbreviated to Phe, Eud-L and HPMC in this report, respectively. The polymeric additives to make the composite particles with drug were comprehensively named "carrier" in this paper. All other chemicals and solvents were of analytical reagent grade, and deionized-distilled water was used throughout the study.

2.2. Manufacturing instruments

The spray-dryer with two-fluid nozzle (SD-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and the spray-dryer with four-fluid nozzle (MDL-050B, Fujisaki Electric, Co., Ltd., Tokushima, Japan) were used in this study. The freeze-dryer (FD-550, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) was used to dry the ice and liquid nitrogen from the resultant composite particles.

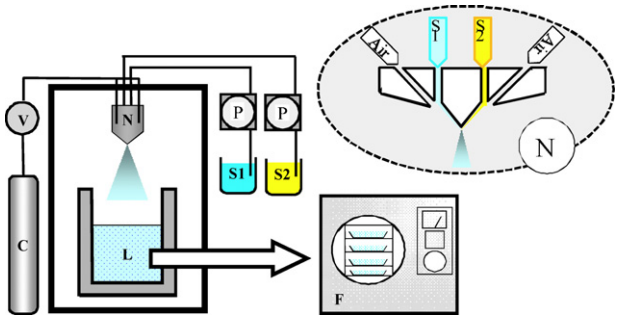

Fig. 1. Schematic diagram of spray freeze-drying apparatus with four-fluid nozzle (4N-SFD) technique. Key: S1: sample solution (1), S2: sample solution (2), P: pump, N: nozzle, L: liquid nitrogen, C: compressor, V: pressure valve, and F: freeze-dryer.

2.3. Preparation of spray freeze-dried composite particles

The whole spray freeze-drying (SFD) apparatus and procedure using four-fluid nozzle (4N) are schematically shown in Fig. 1. Two separated spray solutions were supplied to the nozzle part of spray-dryer at the speed of 10 mL/min each (total feeding speed: 20 mL/min), and the fine mists were atomized by the pressure of compressed air at flow rate of 20 L/min each (total blowing speed: 40 L/min). In the present SFD process, the mists were sprayed to the surface of liquid nitrogen in the stainless steel vessel (6-L volume) instead of air-heated drying chamber as a component of this instrumental unit. The distance between tip of nozzle and the surface of liquid nitrogen was fixed to 20 cm throughout the study. The sprayed mists trapped into the liquid nitrogen were immediately frozen and dispersed in the liquid nitrogen using a magnetic stirrer. After most of liquid nitrogen was evaporated in the ambient condition, the vessel containing the frozen droplets was put into the chamber of the freeze-dryer to sublimate the iced water for over 24 h. The resultant spray freeze-dried (SDF) composite particles were collected and stored in glass vials in a desiccator at room temperature before the characterization measurement.

SFD preparation using conventional two-fluid nozzle (2N), shortly described as "2N-SFD" hereafter, was executed in a same manner as a reference. Because 2N has only one liquid-supplying line, both drug and carrier are necessary to be dissolved in common spray solution in this process. The operational condition of 2N-SFD was same as that of experiments reported in our previous paper (Kondo et al., 2009). In addition, SFD preparation using 4N, shortly described as "4N-SFD" hereafter, was categorized into two types based on the spray pattern as follows: (1) heterogeneous collision method (abbreviated to "4N-SFD-hetero"): drug solution and carrier solution were separately prepared and sprayed, then both solutions were collided and mixed at the tip of the nozzle edge; (2) homogeneous collision method (abbreviated to "4N-SFD-homo"): common spray solution dissolved drug and polymer together was fed to four-fluid nozzle by two liquid-supplying lines, then the solutions having same components were collided. The schematic diagrams of SFD method with three types of spray pattern (2N-SFD, 4N-SFD-homo, 4N-SFD-hetero) were shown in Fig. 2 to help readers' understanding. The representative formulations of spray solution in the 2N-SFD and 4N-SFD-homo methods are tabulated in Table 1. Phenytoin and the carrier were dissolved together in ammonium (NH_3) aqueous solution. The concentration of total solid materials in the spray solution was fixed to 1.0% (w/v) in all formulations. On the other hand, the representative formulations of spray solution in the 4N-SFD-hetero method are tabulated in Table 2. HPMC was dissolved in water instead of NH_3 aqueous

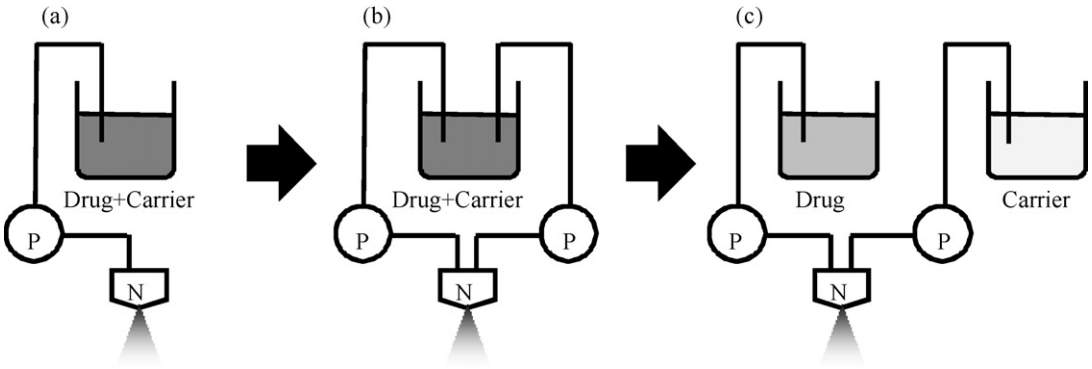

Fig. 2. Schematic diagrams of spray freeze-drying (SFD) method with three types of spray pattern. (A) Single spray method with two-fluid nozzle (2N-SFD), (B) homogeneous collision method with four-fluid nozzle (4N-SFD-homo), (C) heterogeneous collision method with four-fluid nozzle (4N-SFD-hetero). Key: N: nozzle and P: pump.

Table 1
Formulation of spray solution in the 2N-SFD and 4N-SFD-homo methods.

Sample	Spray solution		
	Phe (g)	Carrier (g)	NH_3 aq. (mL)
Phe:Eud-L = 1:1	1.0	1.0	200 (1.0%)[a]
Phe:Eud-L = 1:3	0.5	1.5	200 (1.0%)
Phe:Eud-L = 1:5	0.33	1.67	200 (1.0%)
Phe:HPMC = 1:5	0.33	1.67	200 (1.0%)
Phe only SFD[b]	2.0	–	200 (3.0%)

[a] Numerical values in parenthesis designate the concentration of NH_3 aqueous solution.
[b] Samples of "Phe only SFD" were prepared by both 2N-SFD and 4N-SFD-homo spray methods.

solution. The concentration of total solid materials after mixing both solutions was also fixed to 1.0% (w/v) basically. In order to investigate the effect of solid concentration of spray solution on characteristics of the SFD composite particles, the formulations (Phe:Eud-L = 1:5) with 2.0% and 3.0% (w/v) of the concentration were additionally formulated (see Table 2).

2.4. Morphology and particle size distribution (PSD)

The morphology and particle size of composite particles were observed under a scanning electron microscope (SEM, JSM-6060, JEOL Ltd., Tokyo, Japan). The particles were fixed to the special sample stage and coated using a platinum sputtering equipment (JFC-1600, JEOL Ltd.). The particle size distribution of the composite particles was measured by a laser diffraction scattering method using the diffractometer with the dry dispersing unit (LMS-30, Seishin Enterprise Co., Ltd., Tokyo, Japan). The particles were dispersed into dry air at fixed air pressure of 0.4 MPa. The volume median diameter (D_{50}) was represented as mean particle size. The specific surface area of the particles was measured by a surface area analyzer (Nova-1000, Yuasa Ionics Co., Ltd., Osaka, Japan) using argon gas sorption process. The surface area per powder unit weight was calculated based on the fitting of the adsorption data to the BET equation.

2.5. Crystalline analysis

X-ray powder diffraction (XRPD) analysis was conducted using a Geiger-Flex difractometer (RAD-2VC, Rigaku Co., Tokyo, Japan) with CuKα_1 radiation and a Ni filter at a voltage of 30 kV and a current of 20 mA. Samples were scanned over 2θ range of 5–45° at a rate of 5°/min. Differential scanning calorimetry (DSC) was performed using DSC instrument (DSC-60, Shimadzu Co., Ltd., Kyoto, Japan). Around 5 mg of each test sample was placed in an aluminum pan. The heating program was carried out using a modulated setting at 10 °C/min over 30–310 °C.

2.6. Dissolution study

The dissolution profiles of phenytoin from SFD composite particles were examined with a dissolution tester (NTR-3000, Toyama Sangyo Co., Ltd., Osaka, Japan) using the paddle method according to Japanese Pharmacopeia fifteenth edition (JP15). Sample powders corresponding to 10 mg of the drug were weighed and placed into 900 mL of the dissolution media with holding temperature at 37 ± 0.5 °C. The rotation speed of the paddle was set to 50 rpm in this experiment. The dissolution tests of each sample were performed in both the first fluid (pH 1.2) and second fluid (pH 6.8) defined in the dissolution test of JP15 to estimate the dissolution in the gastrointestinal juice. Aliquots of the solution were withdrawn through the membrane filter (pore size: 0.45 μm) and diluted in methanol to the appropriate concentration. The quantity of phenytoin dissolved was assayed spectrophotometrically at 255 nm by HPLC (LC-10, Shimadzu Co., Ltd.) equipped with an ODS column (RP-18, 5 μm, 4.6 mm × 150 mm, Merck). The phenytoin peak was eluted around 4.5 min when running mobile phase (10 mM KH_2PO_4 solution:acetonitrile, 60:40, v/v) at 1.0 mL/min. Dissolution profiles

Table 2
Formulation of spray solution in the 4N-SFD-hetero method.

Sample	Drug solution		Carrier solution		Solid conc.[b] (w/v%)
	Phe (g)	NH_3 aq. (mL)	Carrier (g)	NH_3 aq. (mL)	
Phe:Eud-L = 1:1	1.0	100 (3.5%)[a]	1.0	100 (0.28%)	1.0
Phe:Eud-L = 1:3	0.5	100 (1.0%)	1.5	100 (0.3%)	1.0
Phe:Eud-L = 1:5	0.33	100 (0.8%)	1.67	100 (0.4%)	1.0
Phe:Eud-L = 1:5	0.67	100 (2.0%)	3.33	100 (0.6%)	2.0
Phe:Eud-L = 1:5	1.0	100 (2.8%)	5.0	100 (1.2%)	3.0
Phe:HPMC = 1:5	0.33	100 (0.8%)	1.67	100 (0%)	1.0

[a] Numerical values in parenthesis designate the concentration of NH_3 aqueous solution.
[b] Concentration of total solid materials in the spray solution after mixed both solutions.

for phenytoin original powder and phenytoin only SFD particles were also studied as references. The dissolution tests of each sample were repeated in three vessels and the average release percentage and the standard deviation were plotted.

3. Results and discussion

3.1. Preparation of SFD composite particles using four-fluid nozzle

The four-fluid nozzle has unique structure with two gas supply lines and two liquid feed passages described in detail in the previous reports (Ozeki et al., 2005; Chen et al., 2006a). It allows two components dissolved in separate solvents to be sprayed. The structure is specially designed that each spray solution fed from two passages are simultaneously atomized by the compressed air and immediately collided and mixed with each other at the tip of nozzle edge. The authors have previously reported the preparation of the composite particles, which were composed of tolbutamide and HPMC as a drug and a carrier, respectively, by SFD method using two-fluid nozzle (2N-SFD) (Kondo et al., 2009). In the current study the four-fluid nozzle was adopted to expand the applications of SFD technique. Furthermore, phenytoin and the enteric methacrylic acid copolymer for pharmaceutical use (Eudragit L100) were newly used as a poorly water-soluble drug and a dissolution modifier, respectively, in addition to HPMC. As a result, the spherical SFD composite particles were successfully prepared with high yield (>90%). It was also found that there was clear difference in the size distribution of SFD particles between both spray patterns. The 4N-SFD method produced the much smaller particles than 2N-SFD method as shown in Fig. 3, which exhibited the size distribution of

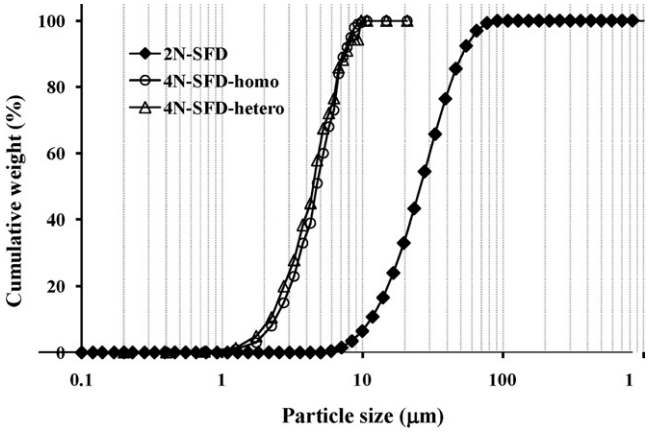

Fig. 3. Particle size distribution profiles of Phe:Eud-L = 1:5 composite particles prepared by SFD method with three types of spray pattern. Key: (square) 2N-SFD, (circle) 4N-SFD-homo and (triangle) 4N-SFD-hetero.

Phe:Eud-L = 1:5 composite particles using three types of spray pattern. The mean particle sizes that are median diameters obtained from size distribution curves are 25.3, 4.72, and 4.45 μm for 2N-SFD, 4N-SFD-homo, and 4N-SFD-hetero methods, respectively. The current 4N developed by the supplier is specifically designed nozzle to produce single-micron sized droplets. The fed solutions were thinly stretched out at the acceleration zone of nozzle edge, and powerfully collided to atomize finely splattered mists. These results are highly consistent with our previous report of spray-dried particles (Chen et al., 2007). Thus, the current 4N-SFD technique was found

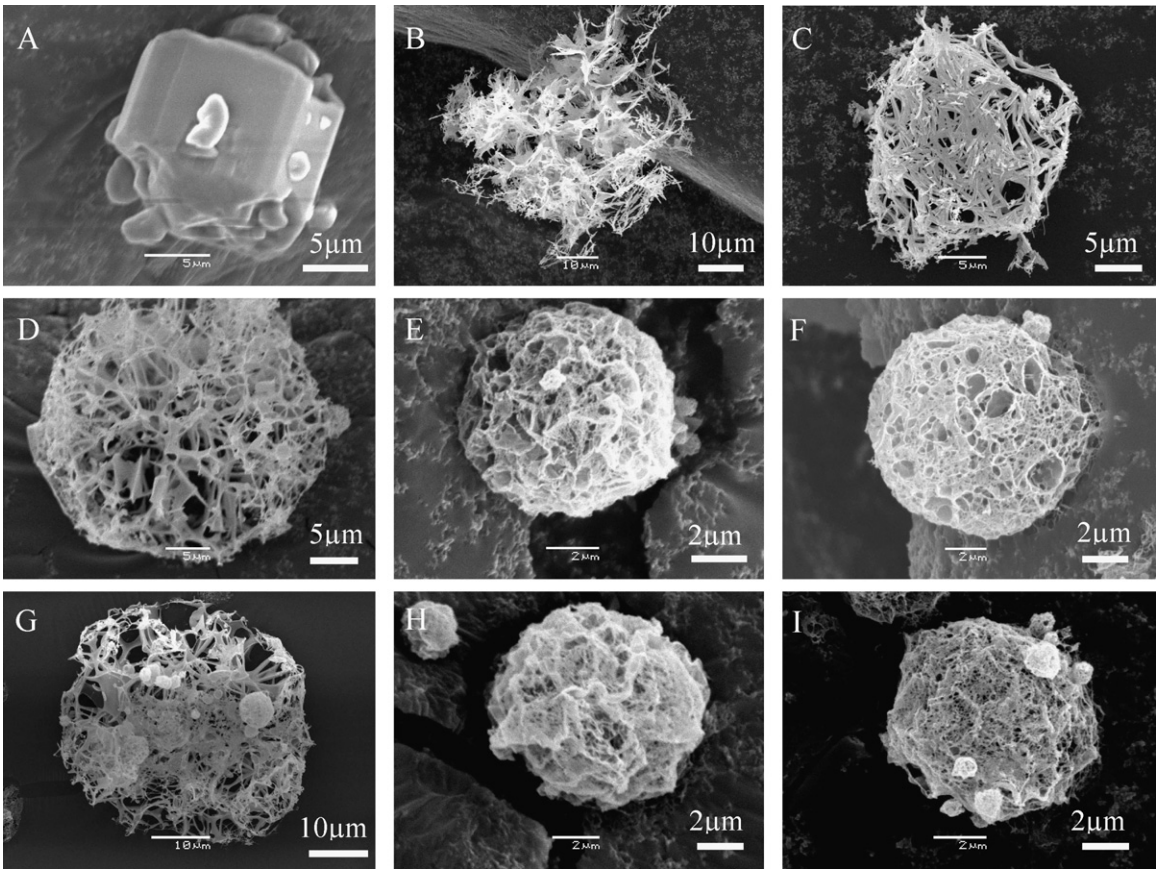

Fig. 4. Scanning electron microphotographs of bulk materials and composite particles prepared by SFD method with three types of spray pattern. (A) Phe bulk; (B) Phe only (2N-SFD); (C) Phe only (4N-SFD-homo); (D) Phe:Eud-L = 1:5 (2N-SFD); (E) Phe:Eud-L = 1:5 (4N-SFD-homo); (F) Phe:Eud-L = 1:5 (4N-SFD-hetero); (G) Phe:HPMC = 1:5 (2N-SFD); (H) Phe:HPMC = 1:5 (4N-SFD-homo); (I) Phe:HPMC = 1:5 (4N-SFD-hetero).

to be effective in realizing the single-micron sized particles in both homogeneous and heterogeneous collision methods.

3.2. Characterization of SFD composite particles prepared by 2N-SFD, 4N-SFD-homo and 4N-SFD-hetero methods

In order to clarify the differentiation of the products obtained by three spray patterns, three types of SFD composite particles were prepared with fixing the ratio of phenytoin against the carrier to 1:5. Either Eud-L or HMPC was formulated in the particles as a carrier. The morphological appearances of each composite particle and phenytoin bulk powder were observed by SEM as shown in Fig. 4. It was found that every SFD particles have common morphological feature having numerous pores both on the surface and inside of the particle. Such internal fine porous structure as a honeycomb is quite specific compared to the conventional spray-dried particles which have smooth surface with no pore (Gaete et al., 2008; Tewa-Tagne et al., 2007). The nano-sized pores were assumed to be a vestige of ice crystals because the sprayed droplets were frozen just after immersing in liquid nitrogen and freeze-dried while keeping their shapes and sizes. The SFD particles composed of only phenytoin with no carrier do not display the spherical mass but coarse fluffy structure like twine of cotton fiber (see Fig. 4B and C). On the other hand, the SFD particles containing the carriers have spherical shape with rigid network in every types of spray pattern (see Fig. 4D–I). The morphological difference just described above means that the polymeric carriers formulated in particles (Eud-L and HPMC) may play the role to form the framework and strengthen the structure. In addition, SEM indicated that the 4N-SFD particles were found to be smaller size and have finer internal structure than the 2N-SFD particles as also shown in particle size distribution (Fig. 3). The satellite particles less than 1 μm in diameter were also observed in SEM photographs of 4N-SFD products, which are assumed to be generated by splattering at collision between droplets. However, no difference in size and morphology between 4N-SFD-homo and 4N-SFD-hetero particles was observed.

The data of specific surface area shown in Table 3 indicated that the present SFD particles have significantly large surface area compared to the bulk material of phenytoin. It should be emphasized that the SFD particles are characterized by such tremendous surface area due to the minute porous structure. Especially, the particles obtained from both 4N-SFD methods (homo and hetero) had 160-times or over greater than bulk material in terms of specific surface area. In addition, the present values over 100 m^2/g were around 10-times larger than those of particles prepared by spray freezing into liquid (SFL) technique previously developed (Hu et al., 2002). The specific surface areas were not different between 4N-SFD-homo and 4N-SFD-hetero methods. The smaller particles prepared from 4N-SFD methods had the advantage of producing the large surface area rather than those from 2N-SFD method. Furthermore, the specific surface areas of SFD particles with phenytoin only were not large as those of SFD particles including phenytoin and carriers as anticipated from morphological viewpoint mentioned above.

Table 3
Specific surface area of bulk materials and composite particles prepared by SFD method with three types of spray pattern.

Sample/spray pattern	Specific surface area (m^2/g)		
	2N-SFD	4N-SFD-homo	4N-SFD-hetero
Phe only SFD	14.5	16.3	–
Phe:Eud-L = 1:5	38.4	126	126
Phe:HPMC = 1:5	50.9	141	129
Bulk materials	Phe	Eud-L	HPMC
	0.77	18.6	0.25

The crystalline property of phenytoin loaded in the SFD composite particles was investigated by X-ray powder diffraction (XRPD) and DCS. XRPD patterns and DSC curves of Phe:Eud-L = 1:5 particles shown in Fig. 5 indicated that the drug was assumed to be amorphous in the particles prepared by every spray methods (2N, 4N-homo, 4N-hetero) since both diffraction peaks and endothermic peak derived from the crystal of the drug were completely disappeared. Whereas, the same crystalline form as original bulk was remained in SFD particles composed of only phenytoin although the crystallinity was somewhat decreased after treatment. In case of HPMC as a carrier, the same results were obtained as Eud-L.

The release property from the SFD composite particles was examined in the media adjusted at pH 1.2 and 6.8 to simulate the environments of the stomach and small intestine, respectively. The release profiles of Phe:HPMC = 1:5 particles presented in Fig. 6 indicated that the release of the drug from every SFD particles was considerably improved in comparison to the phenytoin bulk powder or only Phe SFD particles with no carrier in both media at pH 1.2 and 6.8. The increased release rate of the SFD particles is due in part to the hydrophilic and water-soluble characteristics of HPMC independent of pH. In addition, the minute porous structure result-

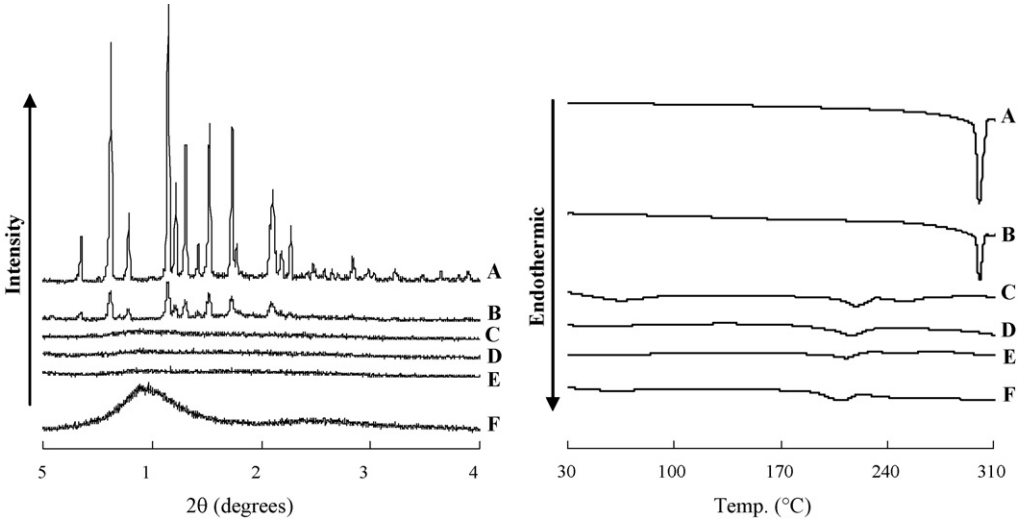

Fig. 5. X-ray powder diffraction patterns (left) and DSC profiles (right) of Phe:Eud-L = 1:5 composite particles prepared by SFD method with three types of spray pattern. (A) Phe bulk; (B) Phe only SFD (2N-SFD); (C) 2N-SFD; (D) 4N-SFD-homo, (E) 4N-SFD-hetero and (F) Eud-L bulk.

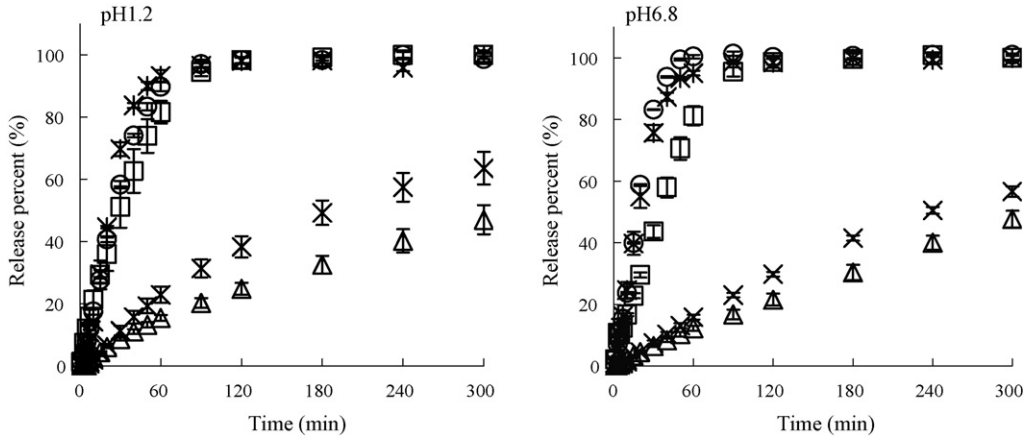

Fig. 6. Release profiles of drug from Phe:HPMC = 1:5 composite particles prepared by SFD method with three types of spray pattern (left: pH 1.2, right: pH 6.8). (×) Phe bulk, (△) Phe only 2N-SFD, (○) 2N-SFD, (□) 4N-SFD-homo and (✳) 4N-SFD-hetero.

Table 4
Specific surface area of Phe/Eud-L composite particles prepared by SFD method with three types of spray pattern.

Sample/spray pattern	Specific surface area (m^2/g)		
	2N-SFD	4N-SFD-homo	4N-SFD-hetero
Phe:Eud-L = 1:1	14.0	99.2	41.0
Phe:Eud-L = 1:3	28.4	105	80.4
Phe:Eud-L = 1:5	38.4	126	126

ing in enhanced surface area and amorphous nature of the drug in the particles also might accelerate the dissolution. However, it was found that the dissolution rate is not directly related to the specific surface area because phenytoin bulk (specific surface area: 0.77 m^2/g) shows a bit faster release than only Phe SFD particles (14.5 m^2/g) even if the surface area is quite different. Only Phe SFD particles were observed to be flocculated in the dissolution media. Therefore, the key factor responsible for improved release behavior is assumed to be the effective surface area caused by good wetting to water. Comparing the profiles between two 4N-SFD particles

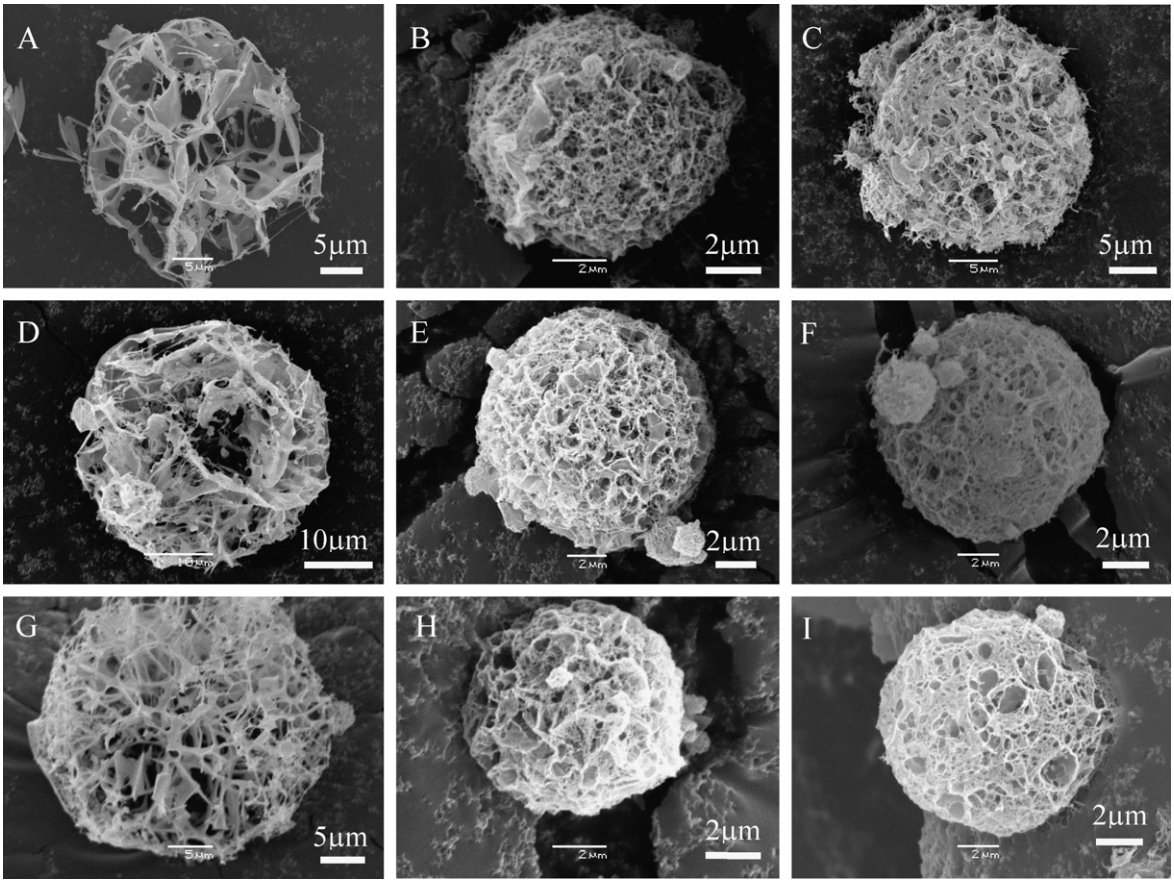

Fig. 7. Scanning electron microphotographs of Phe/Eud-L composite particles prepared by SFD method with three types of spray pattern. (A) Phe:Eud-L = 1:1 (2N-SFD); (B) Phe:Eud-L = 1:1 (4N-SFD-homo); (C) Phe:Eud-L = 1:1 (4N-SFD-hetero); (D) Phe:Eud-L = 1:3 (2N-SFD); (E) Phe:Eud-L = 1:3 (4N-SFD-homo); (F) Phe:Eud-L = 1:3 (4N-SFD-hetero); (G) Phe:Eud-L = 1:5 (2N-SFD); (H) Phe:Eud-L = 1:5 (4N-SFD-homo); (I) Phe:Eud-L = 1:5 (4N-SFD-hetero).

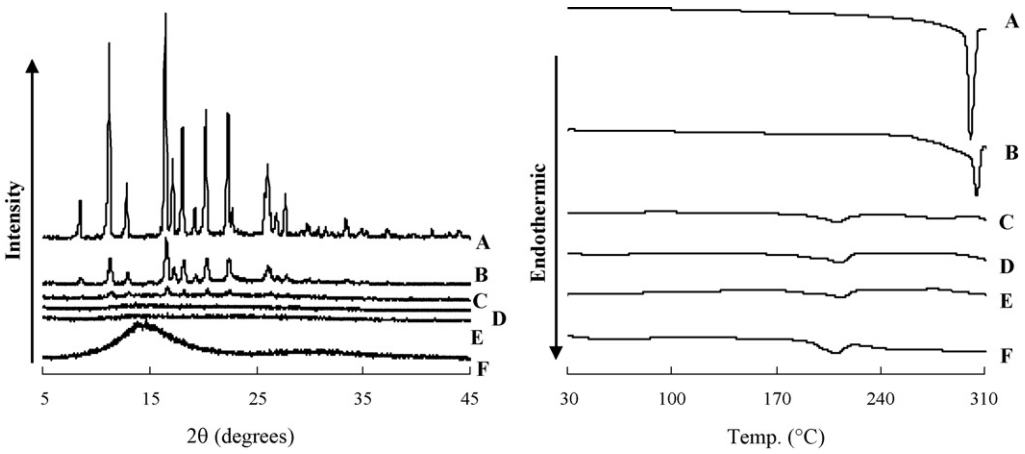

Fig. 8. X-ray powder diffraction patterns (left) and DSC profiles (right) of Phe/Eud-L composite particles prepared by heterogeneous collision method with four-fluid nozzle (4N-SFD-hetero). (A) Phe bulk; (B) Phe only 4N-SFD; (C) Phe:Eud-L = 1:1; (D) Phe:Eud-L = 1:3; (E) Phe:Eud-L = 1:5; (F) Eud-L bulk.

(homo and hetero), the release of hetero particles was a bit faster than that of homo particles. The difference is assumed to be caused by partial segregation of the polymeric carrier inside the particles, but could not be explained in detail. The release profiles of the SFD particles containing Eud-L are presented in the next section.

3.3. Effect of drug–carrier ratio on characteristics of SFD composite particles

The particles having various ratios of Phe:Eud-L were prepared by SFD methods with three types of spray pattern in order to evaluate the influence when increasing the loading of the drug. All SFD particles composed of Phe:Eud-L = 1:1, 1:3, 1:5 were successfully prepared in every spray patterns. The drug content in the particles was within 99–101% range against the theoretical values calculated from the amount loaded in the formulation. SEM photographs in Fig. 7 and the values of specific surface area in Table 4 show the structural difference of SFD particles. With increasing Eud-L ratio, the particles became finer geometrical structure resulting in higher specific area in all SFD methods. The particles with higher carrier ratio constructed the nano-structured pores on surface and inside the particles. As also mentioned earlier, the systemic structural difference suggests that the polymeric carrier strongly contributes toward forming fine network structure in the present SFD particles. The crystalline properties in the particles by 4N-SFD-hetero method evaluated by XRPD and DSC are shown in Fig. 8 as a representative of three spray patterns. No diffraction peak in XRPD pattern and no endothermic peak in DSC curve was observed in Phe:Eud-L = 1:3 and 1:5 particles, whereas small diffraction peaks were detected in XRPD pattern in Phe:Eud-L = 1:1 particles. In addition, the XRPD peaks of 2N-SFD particles were found to be higher than those of 4N-SFD particles with respect to Phe:Eud-L = 1:1. These results suggest that a part of drug still remained crystalline state in SFD particles composed of same amount of phenytoin and Eud-L, but the carrier completely inhibited crystal growth of the drug in the particles with Phe:Eud-L = 1:3, producing amorphous state. Further, the smaller droplets produced by 4N-SFD methods might freeze more rapidly in liquid nitrogen than larger ones by 2N-SFD method, resulting in less crystalline state in 4N-SFD particles. Overhoff et al. (2007) have also demonstrated that rapid freezing is strongly contributed to amorphism of the drug in freeze-drying process.

The release profiles of Phe:Eud-L = 1:1, 1:3, 1:5 particles prepared by three spray patterns were illustrated in Fig. 9 (A: 2N-SFD, B: 4N-SFD-homo, C: 4N-SFD-hetero) in the media adjusted at pH 1.2 and 6.8. Overall, the SFD composite particles with Eud-L showed delayed release compared to the phenytoin bulk powder or only Phe SFD particles with no carrier in the acidic medium. In contrast, the release profiles of the drug from every SFD particles were considerably improved in the medium at pH 6.8. These enteric release behaviors are considered to be attributed to pH-dependent property of Eud-L dissolved more than pH 6.0. Comparing the release profiles among three spray methods, 4N-SFD-hetero particles showed the strongest delayed effect at pH 1.2 and the weakest accelerated effect at pH 6.8. The 4N-SFD particles which have large specific area must be essentially advantageous to improve the dissolution of the drug, but the results were not consistent with such speculation. It is assumed that the effective surface area related to drug release was not so large as values measured by argon gas sorption method because the porous channels might be too narrow to be penetrated by water. The same reason could lead to the contrary result, that is, only Phe SFD particles with larger specific surface area showed slower release than Phe bulk as mentioned earlier. In addition, phenytoin, hydrophobic substance, may be heterogeneously dispersed in the 4N-SFD-hetero particles due to immediate freezing just after mixing between drug and carrier solutions. As a result, the hydrophobic domain scattered on the surface of the particles would inhibit the penetration of dissolution media. In contrast, the 2N-SFD and 4N-SFD-homo particles which might have homogeneous structure with more hydrophilic surface gave the considerable release improvement at pH 6.8, attaining almost 100% release within 10 min.

3.4. Effect of concentration in spray solution on characteristics of SFD composite particles

The composite particles of Phe:Eud-L = 1:5 were prepared in the 4N-SFD-hetero method by increasing the loading of the drug and carrier in spray solution from 1.0% of standard concentration to 2.0 and 3.0%. SEM photographs indicated that the particles became dense with smaller pore size holding the spherical shape and single-micron diameter (Fig. 10). The unique honeycomb structure was not observed in particles prepared from 3.0% of spray solution. As a result, the specific surface area decreased stepwise (Table 5). This

Table 5
Specific surface area of Phe:Eud-L = 1:5 composite particles prepared by heterogeneous collision method with four-fluid nozzle (4N-SFD-hetero).

Sample	Phe:Eud-L = 1:5		
Solid concentration in spray solution	1.0%	2.0%	3.0%
Specific surface area (m^2/g)	126	83.5	66.5

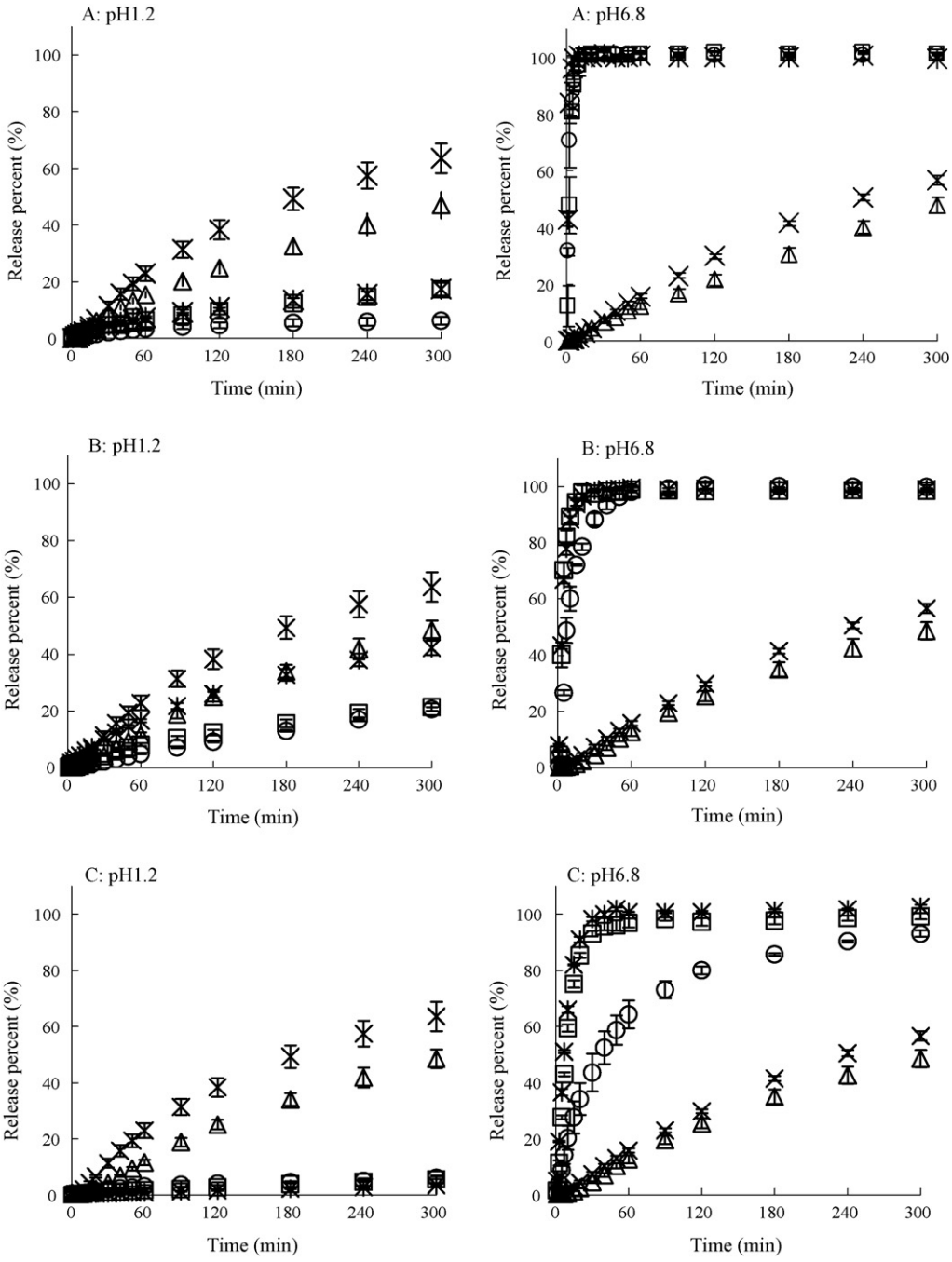

Fig. 9. Release profiles of drug from Phe/Eud-L composite particles prepared by (A) 2N-SFD, (B) 4N-SFD-homo and (C) 4N-SFD-hetero methods (left: pH 1.2, right: pH 6.8). (×) Phe bulk, (△) Phe only (A: 2N-SFD, B and C: 4N-SFD), (○) Phe:Eud-L = 1:1, (□) Phe:Eud-L = 1:3 and (✳) Phe:Eud-L = 1:5.

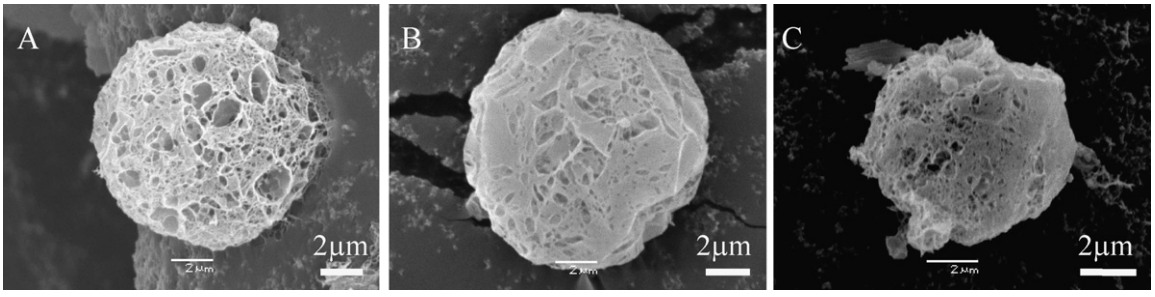

Fig. 10. Scanning electron microphotographs of Phe:Eud-L = 1:5 composite particles prepared by heterogeneous collision method with four-fluid nozzle (4N-SFD-hetero). Solid concentration in spray solution: (A) 1.0%; (B) 2.0%; (C) 3.0%.

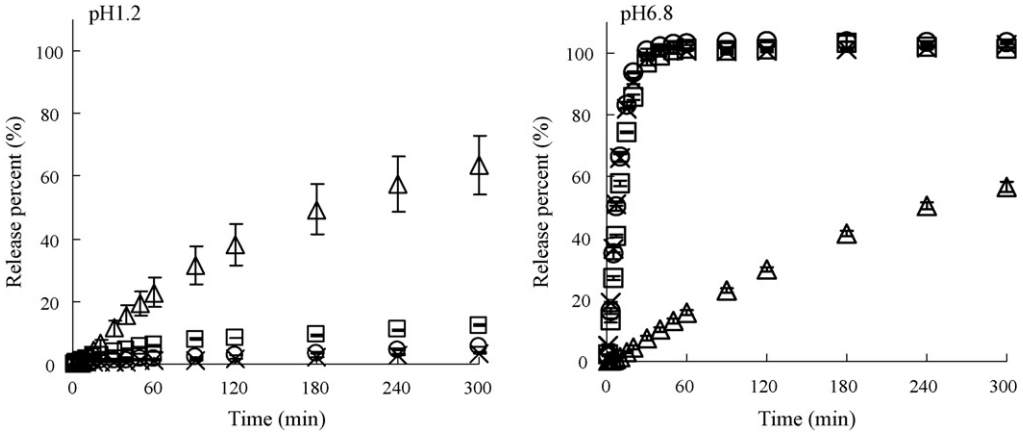

Fig. 11. Release profiles of drug from Phe:Eud-L = 1:5 composite particles prepared by heterogeneous collision method with four-fluid nozzle (4N-SFD-hetero) (left: pH 1.2, right: pH 6.8). Total solid concentration in spray solution: (×) 1.0%, (○) 2.0%, (□) 3.0%, and (△) Phe bulk.

decrease tendency of specific surface area is not consistent with our previous result of 2N-SFD particles of tolbutamide and HPMC, in which the specific surface area became maximum around 9.0% of spray solution (Kondo et al., 2009). This discrepancy in surface area would be attributed to the difference of precipitation process of drug and carrier. Anyway, the specific surface areas of each particle considerably increased having 80-times larger than that of original bulk of phenytoin.

The release profiles showed the typical enteric patterns, that is, delayed in acidic medium and accelerated in neutral pH (Fig. 11). The release rate was almost same among every particle from different spray concentrations. Even if the particles were prepared from the highest spray concentration (3.0%), the rapid release remained in spite of decrease in the surface area.

4. Conclusion

In the present research, the SFD technique, previously developed by authors, was improved further by using the four-fluid nozzle to expand the application of the drugs and carriers. The standard condition for preparation was fixed in 4N-SFD methods of both homogeneous and heterogeneous spray patterns. 4N-SFD methods gave the smaller composite particles with single-micron in diameter and 3–4-times larger specific surface area than the previous 2N-SFD method with holding the quite characteristic porous structure. It was found that phenytoin was dispersed in amorphous state in the polymeric network under one-third of drug loading ratio. 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 technique has enough potential to develop the novel formulation for solubilization of the poorly water-soluble drug. Especially, SFD method with heterogeneous spray pattern could allow drug and dissolution modifier to be dissolved in separate solvents such as aqueous and non-aqueous solvents, overcoming problems with finding and using a common solvent. Our research of 4N-SFD using organic solvents is progressing and will be reported in our following papers.

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