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Distribution of ethenzamide and other ingredients on granule surfaces studied by Raman microspectroscopy and mapping

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Distributions of API and medical additives in granules were analyzed using Raman microspectroscopy and mapping. In order to clearly detect ingredients present at low levels, the characteristic peak for each ingredient was used for identification. Two granulation processes, tumbling granulation and high-shear granulation were selected to examine the feasibility of Raman microspectroscopy for investigating granules. Ethenzamide, lactose monohydrate, cornstarch and methylcellulose were used to make model granules. Methylcellulose was distributed homogeneously from the early stage in both granulation methods. Cornstarch and lactose showed similar distribution properties in high-shear granulation. It was presumed from this observation that similar chemical structures with high-hydrophilic groups in the two compounds determined their similar distribution properties. These results suggest that Raman microspectroscopy using the unique absorption peak of each ingredient can detect each ingredient in the individual pixel size (2×2 μm). This analytical method can contribute to evaluation of granular conditions and granulation processes.

1. Introduction

Granulation is often performed to modify mobilization of materials in the manufacturing process and/or improve tablet manufacturing. Granulation often affects critical pharmaceutical quality attributes such as the dissolution of pharmaceutical products. Securing granule quality is important for product quality control. Parameters such as dissolution, disintegration, and rapidity of osmosis are affected not only by the characteristics of the raw materials but also the surface characteristics of the granules. Especially, it is well known that granules resulting from granulation processes in pharmaceutical manufacturing are greatly influenced by physical factors such as the granule surface or the uniformity of ingredients. In order to characterize the granules for pharmaceutical quality, a microscope is commonly applied. However, microscopic analysis only gives visual information, not chemical information such as the distribution of API and excipients. Therefore, in recent years, chemical imaging/mapping techniques such as IR/NIR imaging have been employed to analyze blending homogeneity and the distribution of ingredients on the surface of pharmaceuticals (Lyon et al. 2002; Reich et al. 2005; Ma and Anderson 2007; Šašić et al. 2007; Shah et al. 2007; Zidan et al. 2007; Awa et al. 2008; Gendrin et al. 2008; Gowen et al. 2008; Hilden et al. 2008; Li et al. 2008). The imaging/mapping techniques generally have the advantage of being able to measure a relatively large domain in a short time. However, it is difficult to measure pharmaceutical products non-destructively by methods such as the IR/NIR imaging because sample preparation including cutting, compression and smoothing of the granule surface should be needed. Contrastively, Raman microspectroscopy needs no sample preparation. Moreover, multivariate data analysis such as PLS analysis is often performed with NIR spectra to extract the chemical information of ingredients because the data obtained by NIR is only the average spectrum of all ingredients in pharmaceuticals. The processing of data by an algorithm for multivariate analysis sometimes has low reproducibility. So, casual use of multivariate analysis has the risk of producing an artifact image. On the other hand, the Raman microspectroscopy can provide the essential chemical information without processing of the multivariate analysis because characteristic peaks that are derived from a chemical structure of ingredient are comparatively distinguishable.

Although studies applying spectroscopy to monitor the granulation process have been performed (Jørgensen et al. 2004; Li et al. 2005; Rantanen et al. 2005; Li et al. 2007; Nieuwmeyer et al. 2007; Abu et al. 2007; Luukkonen et al. 2008), there have only been a few studies analyzing of granule shape and/or properties (Sakamoto et al. 2007; Fujimaki et al. 2007; Papp et al. 2008; Šašić et al. 2008). In this study, the applicability of Raman microspectroscopy and mapping with high specificity was examined for analyses of the surface of granules and for evaluation of the granulation processes.

2. Investigations and results

2.1. Granules produced by the tumbling granulation process

Granules were classified into five fractions by visual observation, and the range of granule size in each fraction

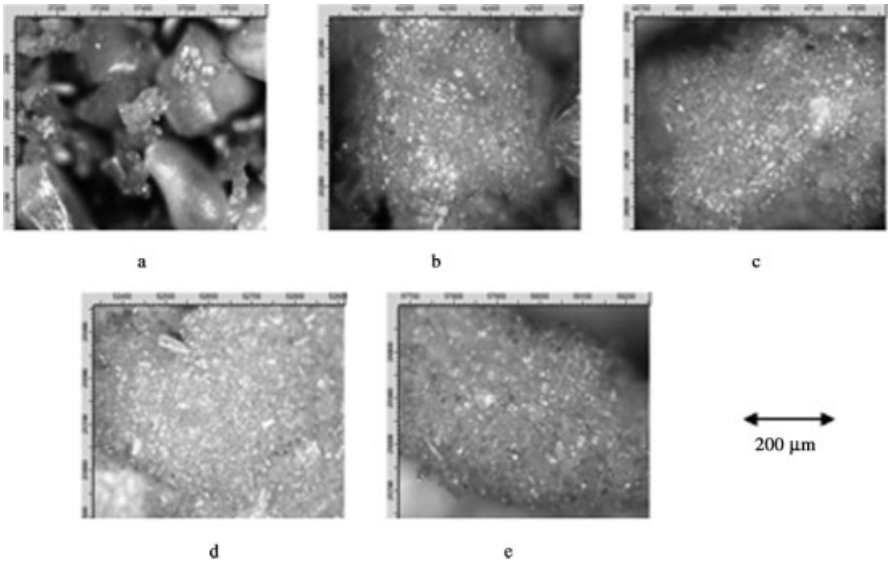

Fig. 1:
Microphotographs of granules obtained by tumbling granulation. Granules were classified into five fractions according to size. The arrow shows the 200 μm length. Granule growth depended on the aggregation of finely powdered ingredients

was classified by microscopy (smaller than 200 μm, 200 to 500 μm, 500 to 1000 μm, 1000 to 1500 μm, 1500 to 2000 μm, and larger than 2000 μm). Microphotographs of granules are shown in Fig. 1. The granulation progress was observed after water was added. Granule size was changed by the aggregation of powders. Almost all observed granule surfaces were very similar in surface appearance although various sizes of granules were seen. This observation suggests that the growth of granules is caused by the aggregation of each ingredient powder.

Microphotographs and Raman spectra of 200−500 μm size particles are shown in Fig. 2. The Raman spectra were obtained from each small lump taking a microscopically-visible characteristic shape on granules (measured view area: 2×2 μm). This observation suggests that distribution of each ingredient granule is distinguishable not only by visual analysis but also by Raman spectroscopy.

In order to examine the distribution of each ingredient, the obtained spectra were compared with the Raman spectra of reference standards. Fig. 3-1 shows optical-microphotographs and Raman spectra of each ingredient. Each ingredient has a distinguishable shape in the microphotograph. Raman chemical maps of granules were obtained using the normalized intensity of the peak (780 cm^{-1} for ethenzamide; 900 cm^{-1} for lactose; 940 cm^{-1} for corn starch; 2830 cm^{-1} for methyl cellulose (Fig. 3-2)) of each compound. The Raman chemical map of granules classified into the 200–500 μm fraction is shown in Fig. 4. Even in the early stage (20 min) of a granulation, methylcellulose was uniformly distributed in the area of the 100×100 μm map. However, all other ingredients were distributed unevenly. The Raman chemical map of granules classified in the 500–1000 μm fraction is shown in Fig. 5. The domain size of each ingredient was smaller than those observed in Fig. 4.

The Raman map of the 1000–1500 μm fraction is shown in Fig. 6. The domain size of each ingredient is still smaller than those observed in Fig. 5 (500 to 1000 μm), and the ingredients contained in the granules were distributed finely.

The domain size of methylcellulose was approximately constant without depending on particle size as those of others in Fig. 4 and Fig. 5. It was considered that methylcellulose was already distributed uniformly in the early stage of the granulation process because of its high dispersibility. On the other hand, it was found that domain

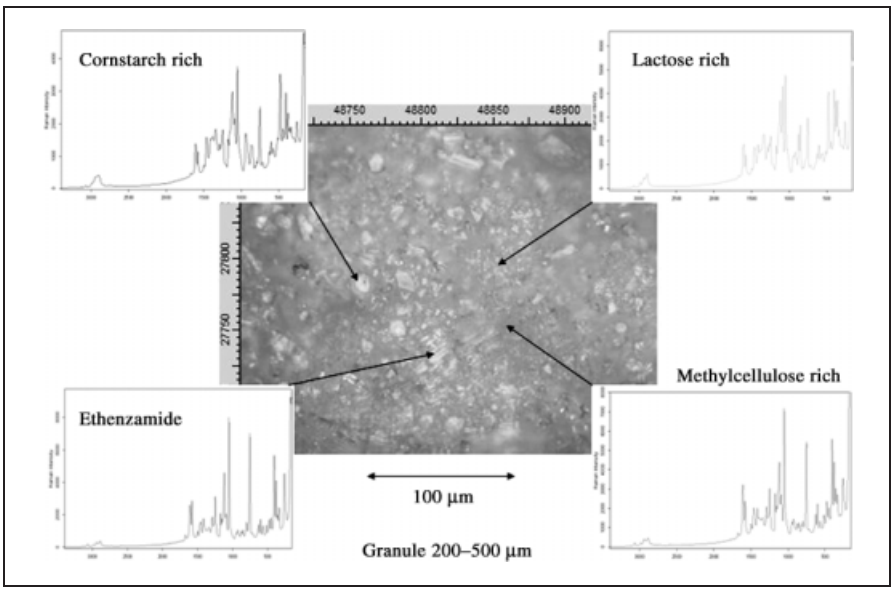

Fig. 2:
Microphotograph of granules classified in the 200–500 μm particle-size fraction. Raman spectra (measured view area: 2×2 μm) of aggregated powders revealed the istinctive shape of each raw material peak even though overlapping of spectra derived from comparably large amounts of ethenzamide was observed

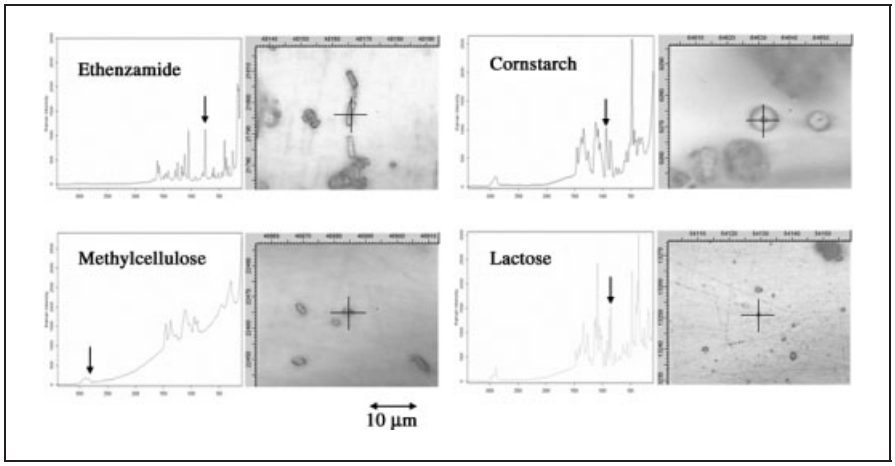

Fig. 3-1:
Micrographs and Raman chemical maps of each ingredient. Distinctive shapes of each ingredient are observed in the microphotograph. The arrow points to a unique peak that does not overlap with those of other ingredients in the Raman spectrum

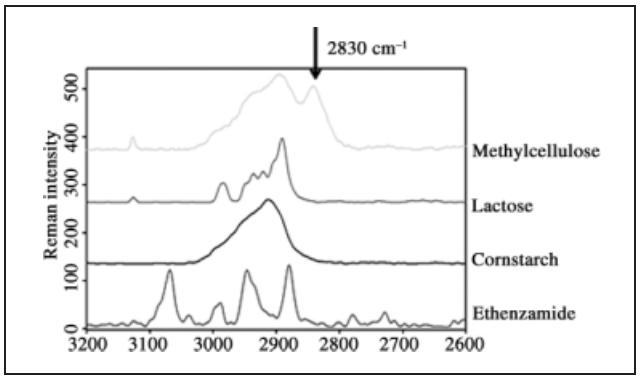

Fig. 3-2: Closeup view of the Fig. 3-1 (specific peak of methylcellulose; range of 3200–2600 cm^{-1})

sizes of three other ingredients became smaller as the particles became bigger, ultimately showing fine distribution on the granule surface. It was considered that useful information about the distribution of ingredients on the granules could be provided because the method had detectability for a distribution of a small amount of ingredient (about 7%) such as methylcellulose. In this experiment, ethenzamide, which has a relatively large domain size of about 20–50 μm, was distributed unevenly, like an island in the sea.

2.2. Granules produced by high-shear granulation

Microphotographs of granules that were sampled 10 min after the process was started are shown in Fig. 7. It was difficult to find a characteristic part from each ingredient domain microscopically.

In order to investigate the distribution of each ingredient, Raman chemical maps were obtained using the normalized intensity of the peak (780 cm^{-1} for ethenzamide; 900 cm^{-1} for lactose; 940 cm^{-1} for corn starch; 2830 cm^{-1} for methyl cellulose) of each compound as a procedure similar to that used in Figs. 4, 5, and 6. Raman mapping images of granules at 3 min, 5 min, and 10 min of granulation time at 40 rpm are shown in Fig. 8. The distribution of each ingredient in the measured area (100×100 μm) was obtained using the Raman mapping technique. It was revealed that methylcellulose was uniformly distributed while the other ingredients – ethenzamide, lactose and cornstarch – were distributed heterogeneously. This observation was almost the same as that of the tumbling granulation. The domain size of ethenzamide in the high-share granulation became smaller than those of the tumbling granulation, and the number of domains was increased. This result suggests that the mechanism of high-shear granulation would contribute to distributing more minute lumps of ethenzamide powder onto the granule surface than the tumbling granulation used in this study. Lactose was gradually and uniformly distributed

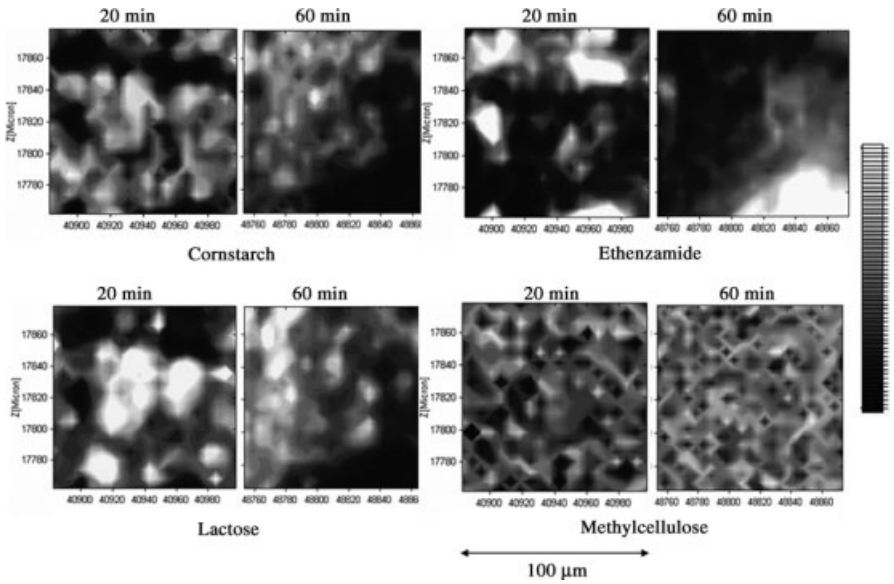

Fig. 4:
Raman chemical maps of all ingredients on the granule (classified in the 200–500 μm particle-size fraction). Methylcellulose was distributed homogeneously from the early stage of granulation

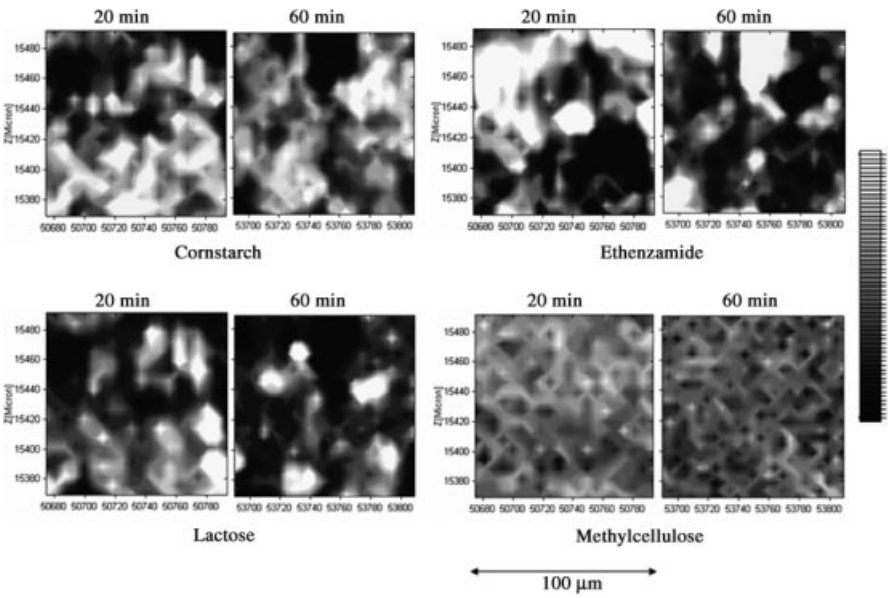

Fig. 5:
Raman chemical maps of all ingredients on the granule (classified in the 500–1000 µm particle-size fraction). The domain size of each ingredient on the Raman chemical maps is smaller than those observed in Fig. 4

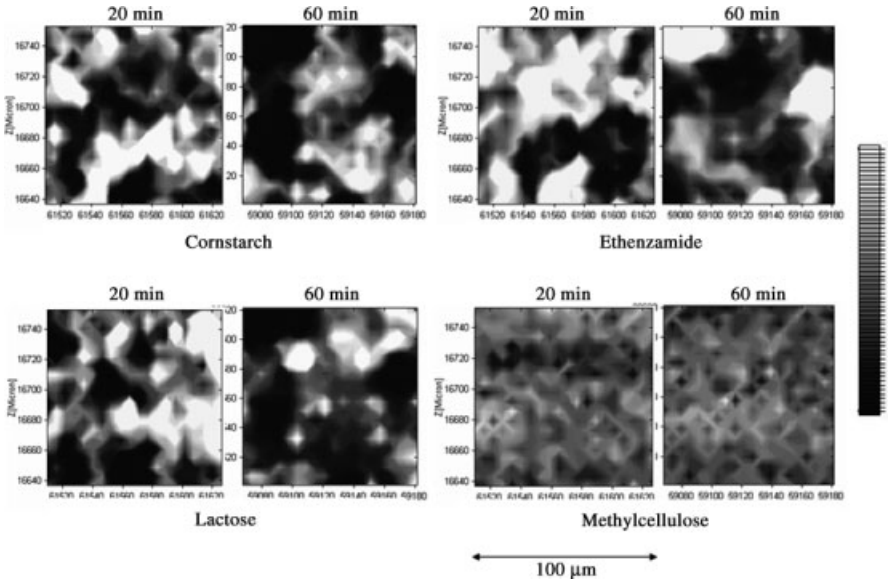

Fig. 6:
Raman chemical maps of all ingredients on the granule (classified in the 1000–1500 µm particle-size fraction). The domain size of each ingredient on the Raman chemical maps is smaller than those observed in Fig. 5

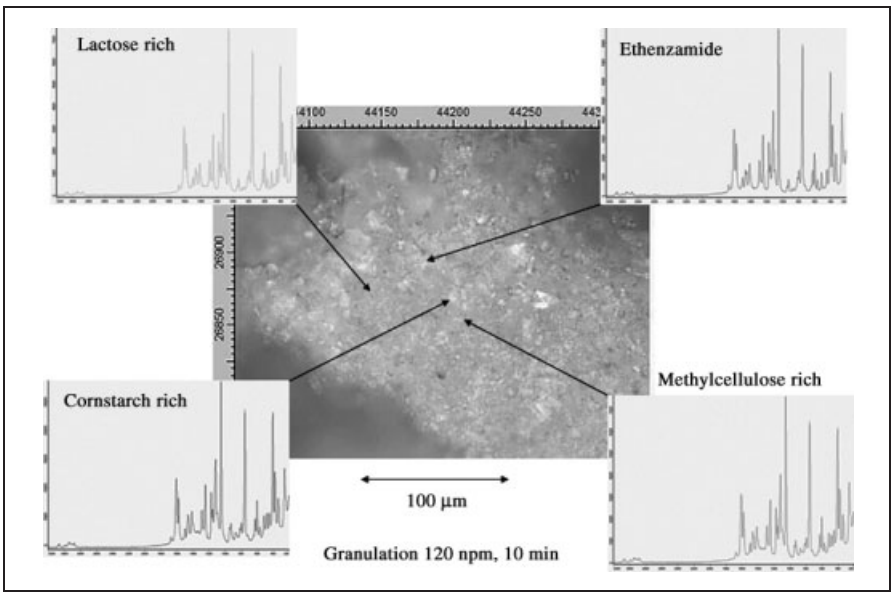

Fig. 7:
Microphotograph of granules produced by high-shear granulation and Raman spectra of some parts of granules. All parts of spectra show similar features, but characteristic peaks derived from each ingredient were detected in each spectrum

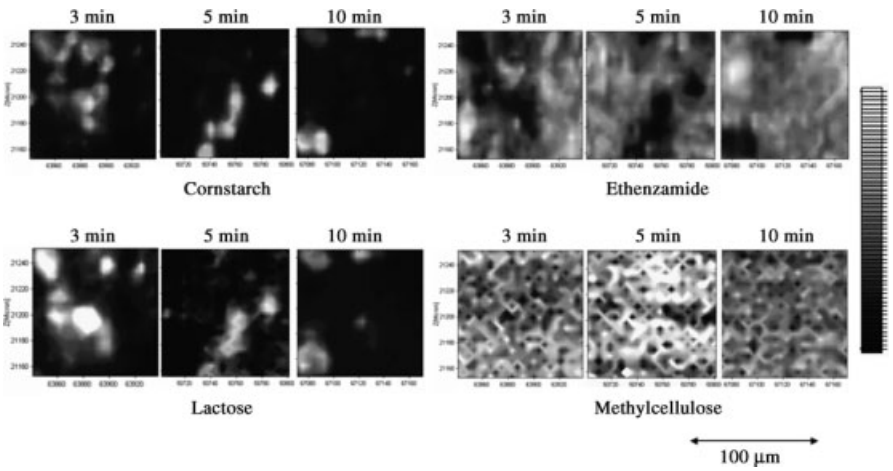

Fig. 8:
Raman chemical maps of granules (at 40 rpm). Methylcellulose was homogeneously distributed at every sampling time of the tumbling granulation

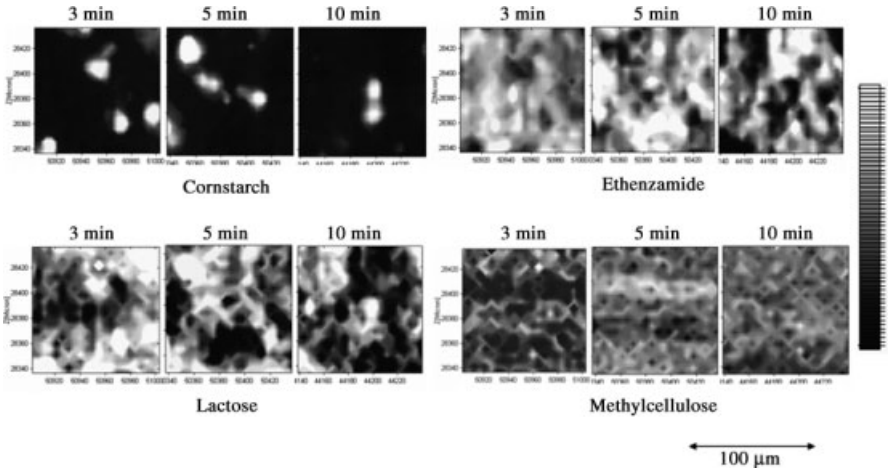

Fig. 9:
Raman chemical maps of granules (at 120 rpm). All ingredients seemed to be almost evenly distributed in the observation areas at every sampling time. Although overlapping between cornstarch and lactose areas was observed, the distribution profile of lactose was ifferent from that of cornstarch

with the passage of time; this phenomenon suggests that a longer time would be needed to achieve fine distribution of this compound than of ethenzamide. Overall, these findings suggest that the distribution states of ingredients are influenced according to not only the aggregation characteristics of each ingredient, but also the type of granulation method or granulation time.

On the other hand, the distribution states of cornstarch and lactose gradually tended to overlap each other. It was assumed that this phenomenon, which was not observed in granules obtained by tumbling granulation, was caused by an interaction such as a kind of affinity based on hydrophilic molecular structure between the two compounds. It was also assumed that those molecular structures have a higher affinity (hydrophilic) than other ingredients, because cornstarch and lactose are saccharides. Therefore, these ingredients will tend to show similar distribution properties when each ingredient is finely distributed. Figure 9 shows the Raman chemical maps of granules sampled at 3 min, 5 min and 10 min of the granulation process at 120 rpm. All ingredients seemed to be almost evenly distributed in the observation areas at every sampling time. Although the overlap between the cornstarch and lactose areas was observed, lactose was homogenously distributed with the passage of time. This observation suggests that the high-shear granulation process with high rotation speed gives a homogeneous distribution of lactose compared with the process performed at the low rotation speed. Heterogeneous distribution of lactose on granules might be explained by combination of the three processes, which are the granulation and the distribution, the re-distribution, in a high-share granulation process. Further investigation of this phenomenon is needed.

2.3. Distribution properties at granulation process

Methylcellulose has been homogeneously distributed at the early stage of the both granulation process in this study. Other ingredients were distributed finely. From these findings, it was considered that the growth of granules and the distribution of each ingredient were progressed together. The domain sizes of ethenzamide in high-share granulation became smaller than those of the tumbling granulation, and the number of domains was increased. This indicates that the high-share granulation is suitable to give fine distribution of ethenzamide than the tumbling granulation. Moreover, in the case of the high-share granulation, the distribution time up to homogeneity of lactose seemed to be longer than in the case of ethenzamide. Not only the granulation method but also a kind of ingredients would give big differences among distribution profiles of ingredients.

Moreover, the distribution properties between cornstarch and lactose had the tendency of overlapping each other gradually. Thus, it is suggested that the interactions of ingredients affected their distribution properties. However, the distribution attribute between lactose and cornstarch was different at 120 rpm which was a comparatively high rotation speed. From these finding, the possibility that the granulation conditions influenced the interaction of each ingredient which gives the influence to the distribution

properties was suggested. When the granulation time is prolonged, the average particle diameter generally increases by slow aggregation of particles, and the aggregates of raw materials are getting bigger.
The growth of granules and the distribution of each ingredient progressed simultaneously, and the distribution profile of each ingredient could be observed by Raman microspectroscopy.
These results suggest that methylcellulose is distributed on the granule surface quickly. The other ingredients are gradually distributed on the granule surface depending on granulation method, granulation conditions, interactions of each ingredient, and so on.

3. Discussion

The ingredient distributions on the granule surface could be specifically analyzed by Raman microspectroscopy. Using the Raman microspectroscopic analysis, it was possible not only to observe the granule surface condition microscopically but also to investigate the chemical distribution of each ingredient. It was considered that this analytical method was a useful technique that can reveal the distribution states of ingredients. In addition, Raman microspectroscopy was able to analyze the granule surface without sample preparation as is required with NIR chemical imaging.
Although a multivariate analysis has often been used in recent chemical imaging analyses, processed imaging data is commonly influenced by the condition of the sample surface. The Raman microspectroscopic mapping technique used in this study hardly influenced the surface condition, and can show not only visual information but also high-specificity chemical images of each ingredient. Furthermore, visual information consists of chemical information. From these findings, changes in the granule surface state during the granulation process could be analyzed by Raman microspectroscopy using characteristic chemical data. It was considered that this method could be applied to analyzing various physicochemical characteristics of granules. In this study, the authors show the distribution of four kinds of ingredients on the surface of granules. Especially, unique absorption of ingredients constituting less than 4% of the whole could be detected.
The accumulation of fundamental physico-chemical information that is obtained using this innovative analytical technology will also be useful for further understanding of not only product quality but also the pharmaceutical manufacturing process. Moreover, use of this innovative analytical technique as a process analytical technology (PAT), could enable the adoption of the concept of continual improvement in quality systems in the future.

4. Experimental

4.1. Materials

Ethenzamide was purchased from Wako Chemical Co. (Osaka, Japan). Lactose monohydrate, cornstarch and methylcellulose were purchased from Wako Chemical Co. (Tokyo, Japan). All compounds were used without further purification.

4.2. Tumbling granulation

Twenty-five g of ethenzamide, 13.6 g of lactose monohydrate, 7.9 g of cornstarch, and 3.5 g of methylcellulose were put into a rotary mixer, and 10.0 g of purified water was added. The rotation speed and time of the mixer were set at 100 ppm and 40 min, respectively. Granules were dried for 24 h in a vacuum desiccator under reduced pressure.

4.3. High-shear granulation

In order to evaluate the distribution of API, granules containing 70.0% ethenzamide were prepared. Then, 70.0% ethenzamide, 18.6% lactose monohydrate, 7.9% cornstarch and 3.5% methylcellulose were added into a 5 kg-volume high-shear mixing granulator, and this mixture was blended with water (1100 g). The blade rotation rate was set at 120 rpm, and granule samplings were carried out at 3 min, 5 min and 10 min of granulation time. Granules were dried in a shelf-type dryer for 12 h.

4.4. Measurements

All microspectroscopic data were obtained by SENTERRA (Bruker Optics K.K., Tokyo, Japan). The instrument was controlled with OPUS Ver. 6.0 (Ettlingen, Germany), which also processed the data. Excitation wavelength, laser power, integration time, number of scans, and grating were set at 785 nm, 100 mW, 10 s, 1 scan, and 400 lines/mm, respectively.
Granules were classified into five fractions by visual observation, and the range of granule size in each fraction was classified by microscopy (smaller than 200 μm, 200 to 500 μm, 500 to 1000 μm, 1000 to 1500 μm, 1500 to 2000 μm, and larger than 2000 μm). A representative granule was pushed lightly onto a glass slide, and the Raman spectra of small domains of the granule and the whole granule were measured. Raman microspectroscopic measurements were performed for several characteristic regions of a granule.

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