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Verification of the mixing processes of the active pharmaceutical ingredient, excipient and lubricant in a pharmaceutical formulation using a resonant acoustic mixing technology

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Mixing processes are important for making high-quality pharmaceutical formulations and are related to dissolution and chemical stability in pharmaceutical manufacturing. The resonant acoustic® mixing (RAM) technology is a blending method, and it has been reported that it has a unique mixing action for various samples. In this study, in order to apply the RAM method to the pharmaceutical blending process, optimization of the operating conditions of RAM (acceleration and frequency) was conducted by numerical simulation. Powder mixing experiments were carried out using various RAM conditions and also a modified V-shaped mixing device with a powder material of theophylline powder and lactose or magnesium oxide and lactose. The angle of repose of the mixed powder sample was measured as an index of powder flowability and also the degree of powder mixing. A drug uniformity test of the mixed powders was performed to measure theophylline content using high-performance liquid chromatography. The results of these experiments indicate that the optimum values for acceleration and frequency in RAM mixing are 90–100 G and approximately 60 Hz, respectively, which prove the superiority of the RAM method over the ordinary mixing method. The RAM method was estimated to throw the powder upward into the air and perform mixing by utilizing free-fall, possibly by inducing a weightless state without depending on the density and mass of the sample. Therefore, RAM may be applicable to pharmaceutical manufacturing processes.

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1 Introduction

Mixing processes in pharmaceutical manufacturing have a significant impact on formulation characteristics, such as dissolution and stability, through the distribution of functional additives such as lubricants, binders, and disintegrating agents. In the case of solid pharmaceutical formulations, the tablet compression, disintegration, and dissolution properties of tablets vary significantly depending on the mixing characteristics of the raw material powders, and this is known to affect the stability and bioavailability of the formulation.[1–4] Some powders are discontinuous-solids with high flowability, similar to a fluid, when moving. However, powders with wide particle size distributions and high hygroscopicity show low-flowability and are difficult to mixed uniformly, therefore, powder mass handling is not straight-forward.[5,6] V-Blenders, tumbler blenders, ribbon blenders, high shear mixers and fluid-bed systems are used as ordinary mixing systems. The mechanisms and performance of these blenders have been investigated and reported in the literature.[7–13] However, there are many problems with ordinary mixing methods, including long time periods for uniform drug mixing in a low-concentration formulation; particle destruction by the mixing impeller; difficulty in cleaning validation because of complex mixing device structures; and preventive management against leakage of active pharmaceutical ingredients (APIs) with high pharmacological activity.[14,15] Furthermore, because any ordinary mixing method is affected by its mixing conditions, such as device capacity and sample mass, there is a need for reconfirmation of condition settings when scaling up the mixing process to the actual production level.

On the other hand, resonant acoustic® mixing (RAM, Resodyn, MT, USA) technology is a mixing system, which is performed by controlling vibration through acceleration and frequency.[16] It has been reported that powder samples mixed by longitudinal vibration and vortexes are generated in various locations in the container, as shown in Fig. 1.[17–21] In this case, the frequency was set to approximately 60 Hz in order to

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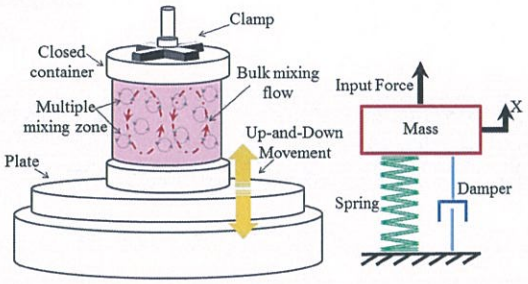

Fig. 1 Reported mixing mechanism of the resonant acoustic mixing (RAM) technology.

transmit the force directly to the mixing plate.[20,21] Vibration motion by the plate's up-and-down movement occurs after the container is fixed by the clamp and the switch is turned on. At this time, multiple mixing zones and bulk mixing flow occur in the container.[20,21] Since it is possible to move powder samples with this method without loss of energy, high-viscosity suspensions can be mixed rapidly and uniformly. In addition, because it mixes uniformly while reducing the generation of heat, RAM is expected to be useable for a wide variety of material powders. However, its basis and mixing mechanism have not been reported. Therefore, it is necessary to examine the optimal mixing parameters and the mixing mechanism of RAM in order for its application in the blending process. Particularly, many pharmaceutical samples are often extremely sensitive, thus their examination is important.

In this study, numerical simulations of amplitudes of the container, vibration velocities and heights of the thrown powder with changes in the RAM parameters (acceleration and frequency), parameter optimization based on numerical simulations and non-invasive verifications of the mixing samples by the RAM method were carried out. Also, a comparative test of the mixing efficiency with an ordinary method using a modified V-shape blender and RAM was carried out in the low-concentration formulation mixing process. We examined the mixing mechanism of RAM in order to verify its usefulness and application in the blending process of pharmaceutical powders.

2 Experimental

2.1 Materials

Lactose monohydrate (DFE Pharma, Goch, Germany) Respitose® SV010 (d_{50}: 105 μm), Respitose® SV003 (d_{50}: 60 μm), Respitose® ML006 (d_{50}: 17 μm) and Lactohale® 100 (d_{50}: 125–145 μm) were used as excipients. Magnesium oxide (MgO, 3 μm, Wako, Osaka, Japan) was used as a lubricant. The API used was theophylline anhydride bulk powder (Shizuoka Caffeine, Shizuoka, Japan). All other chemicals were obtained commercially and were of analytical grade. Milli-Q water was obtained using a Millipore system (Millipore, Billerica, MA, USA) and was used for all samples.

2.2 Numerical simulation by changing RAM parameters

When performing powder mixing, the amplitudes of the container are dependent on the change in frequency and acceleration of RAM. Velocity, which is given by eqn (2), was obtained by differentiating the simple harmonic oscillation of eqn (1). Acceleration, which is given by eqn (3), was obtained by further differentiating eqn (2). Where, x is distance, ω is angular frequency, $\omega t + \alpha$ is phase, α is the first phase, and A is the amplitude.[22]

$$x = A\cos(\omega t + \alpha) \tag{1}$$

$$\frac{dx}{dt} = -\omega A\sin(\omega t + \alpha) \tag{2}$$

$$\frac{d^2x}{dt^2} = -\omega^2 A\cos(\omega t + \alpha) = -\omega^2 x \tag{3}$$

Frequency can be expressed by the angular frequency from eqn (4).[22]

$$\omega = 2\pi f \tag{4}$$

Therefore, the amplitude can be calculated by changing the frequency and acceleration from eqn (5).[22]

$$x_{max} = \frac{\ddot{x}}{(2\pi f)^2} = A \tag{5}$$

Then, the powder is mixed by changing the frequency and acceleration of the RAM. The change in the force applied to the particles during mixing can be predicted. Velocity, which is given by eqn (6), is calculated by from eqn (2) and (5). Where, V_{max} and $-V_{min}$ are equal.[22]

$$V_{max} = \omega A = \frac{\ddot{x}}{2\pi f} \tag{6}$$

Next, the toss height of the powder particles when performing RAM was predicted in constant conditions (frequency and acceleration and the mixing vessel capacity). High tidemark, h, is calculated from eqn (7) for the vertical upward projection.[22]

$$h = \frac{V^2}{2g} = \frac{\left(\frac{\ddot{x}}{2\pi f}\right)^2}{2g} \tag{7}$$

2.3 RAM parameters optimization

2.3.1 Samples preparation by RAM. Powder sample formulations consisted of 84.6% lactose SV010, 14.9% lactose ML006, and 0.5% MgO. After weighing each sample, a total powder weight of 12.6 g was placed in a screw stainless steel can (Shimizu Akira, Niigata, Japan, 150 mL). The RAM equipment was a Resonant Acoustic LabRAM (Resodyn, MT, USA). The screw can was set on the RAM, and powder samples were mixed at a constant frequency of 60 Hz for 20–40 s with a strength of 20–93 G. In this case, the frequency was set to approximately 60 Hz in order to transmit the force directly to the mixing plate.[20,21] The powder samples were mixed with various mixing intensities

(acceleration and time) and they were labeled as 23 G–20 s, 61 G–20 s, 93 G–20 s, 23 G–40 s, 61 G–40 s, and 93 G–40 s. It should be noted that 1.0 G = 9.8 m s^{-2} in this report.

2.3.2 Flowability by angle of repose measurement. Angle of repose measurement is generally used to evaluate the flowability of powder samples. The injection method has been reported to have the highest availability for these types of samples,[23] however it needs more than 10 g of powder sample. Therefore, in this study to measure the angle of repose using smaller amounts of powder samples, a modified gradient method[23] was used. Approximately 30 mg of sample was allowed to stand in a mound. The angle of the mound sample collapsed by the inclination, and the angle was measured as the angle of repose (internal flowability). Fig. 2 shows the modified gradient method instrument. Then, the angle data was compared with that from the commonly used injection method. The relationship between the data from the modified gradient method and injection method showed a straight line ($R^2 = 0.8835$, $n = 90$). Measurements were performed 30 times, and the arithmetic mean (mean) and standard deviation (SD) were calculated.

2.4 Non-invasive verification of the mixing samples by the RAM method

2.4.1 Samples preparation by RAM. Lactose SV010 (LA1) and SV003 (LA2) samples of 12.0 g were weighed and then placed in a stainless steel screw can (Shimizu Akira, Niigata, Japan, 150 mL). The screw can was set on the RAM (2.3.1), and mixed at 100 G–150 s at a frequency of 60 Hz.

2.4.2 Morphology and particle size distribution. Before and after mixing, the particle morphology was observed using a scanning electron microscope (SEM). The SEM equipment was a JSM-6510LV (JEOL, Tokyo, Japan). The SEM equipment was set at the following conditions: acceleration voltage of 15 kV, magnification ×100, working distance of 10 mm, and low vacuum mode. The samples were transferred to carbon tape and carbon coated order to reduce the charge-up phenomenon. In addition, Feret's diameter was measured from the SEM images using an image analysis software (Image-Pro Plus, Media Cybernetics, MD, USA). The particles were 100 count for each sample, in which the overlapping particles were not counted. Measurements were performed 3 times.

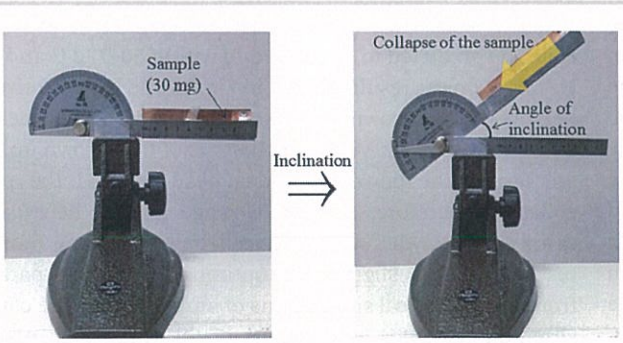

Fig. 2 Modified gradient method instrument used to measure a small amount of powder.

2.5 Comparative tests of mixing efficiency with the ordinary method using a modified V-shape blender method and RAM method

2.5.1 Cryogenic milling of the active pharmaceutical ingredient powder. Niwa *et al.*[24,25] reported that nanoparticles of API were obtained by cryogenic grinding in liquid nitrogen. API bulk powder (20 g) was poured into a 500 mL stainless steel pot with a cork-cap containing liquid nitrogen (−196 °C) and 900 g of zirconia beads (0.50 mm in diameter). A tornado-blade (diameter 75 mm) was rotated for 1 h at a speed of 800 rpm. Liquid nitrogen was added from time to time to maintain the sample at an ultra-low temperature. A cover was placed on the entire equipment in order to prevent condensation in the cup. After grinding, the powder samples were separated from the zirconia beads using a sieve. The morphology of the API after grinding was observed using SEM with magnification ×1000, high vacuum mode, and the other conditions were the same as in 2.4.2.

2.5.2 Specific surface area measurement. Surface area measurement (S) of the powder samples was performed based on the Brunauer–Emmett–Teller (BET) theory. S was measured by the gas flow method (BET 1 point method) using a Monosorb® (Quantachrome, Kanagawa, Japan). The carrier gas was 30% N_2 + 70% He at a flow rate of 15 mL min^{-1} and the drying temperature was 110 °C. In addition, particle size (d, μm) was calculated using eqn (8) and (9) based on the specific surface area (S_w, m^2 g^{-1}). The density (ρ) of the API was 1.47 g cm^{-2}. Measurements were performed 3 times, and the mean was calculated.

$$S_w = \frac{S}{w} \tag{8}$$

$$d = \frac{6}{\rho S_w} \tag{9}$$

2.5.3 Samples preparation by ordinary method using a modified V-shape blender and RAM. Micronized API produced by the cryogenic milling method was used. Powder sample formulations consisted of 98.0% lactose Lactohale® 100 and 2.0% API. After weighing each sample, the total powder weight (40.0 g) was placed in a stainless steel screw can (Shimizu Akira, Niigata, Japan, 150 mL). As an ordinary mixing method using a V-blender, a modified V-shape blender method with a small volume (150 mL) was made using a ball mill rotating gantry (AN2-10S, Nitto Kagaku, Nagoya, Japan) and the screw can was fixed at an angle of 45°. Fig. 3 shows the modified V-shape blender. After the sample was set on the modified V-shape blender, it was mixed at 30 rpm for 0.5 h (C1) and 30 rpm for 10 h (C2). Also, the screw can was set on the RAM (2.3.1) and mixed at 100 G for 0.01 h (C3) and 100 G for 0.03 h (C4) at a frequency of 60 Hz.

2.5.4 Morphology of mixed powders. In order to confirm the uniform dispersion of the API, a chemical structure analysis was performed by mapping using the attenuated total reflection (ATR) method. ATR can be measured non-destructively with a spectrum of IR light to access powder samples in contact with

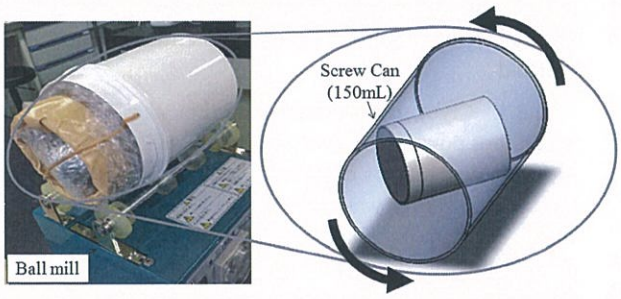

Fig. 3 Modified V-shape blender method as the ordinary mixing method.

the prism.[26] The IR imaging equipment consisted of a Frontier (PerkinElmer, MA, USA) instrument with a Fourier transform infrared spectrometer (FT-IR) and Spotlight 400 (PerkinElmer, MA, USA). Spectrum IMAGE (PerkinElmer, MA, USA), Spectrum Quant (PerkinElmer, MA, USA), and Pirouette® 4.5 (Infometrix, WA, USA) softwares were used. For single reflection ATR measurements, the resolution was 4.0 cm^{-1}, measurement range was 4000–400 cm^{-1}, eight integrations were used, the ATR crystal was diamond/KRS-5, incident angle was 45°, and the background was air for FT-IR. IR spectra were normalized because this easy comparison clarifies the difference between them. The conditions for ATR imaging measurements were as follows: the resolution was 8.0 cm^{-1}, measurement range was 4000–680 cm^{-1}, single integration was used, the ATR crystal was Ge, pixel size was 1.56 μm × 1.56 μm, measurement area was 400 μm × 400 μm, 65 536 spectra were acquired, and the background was air.

2.5.5 Evaluation of flowability by angle of repose measurement. A total of 30 mg of standard powder sample was employed to measure the angle of repose using the modified gradient method (2.3.2). Measurements were performed 30 times, and the arithmetic mean and SD were calculated.

2.5.6 Quantitative sample analysis by high-performance liquid chromatography. The APIs content uniformity in the bottle was quantified *via* high-performance liquid chromatography (HPLC). An LC-10vp series HPLC (Shimadzu, Kyoto, Japan), CLASS-VP 6.14SP1 analysis software (Shimadzu, Kyoto, Japan) and an Inertsil® ODS – 3.5 μm 4.6 × 250 mm column (GL Sciences, Tokyo, Japan) were used. The measurement details were as follows: the detector was an ultraviolet absorption spectrophotometer (measurement wavelength: 228 nm), the quantitative method used absolute calibration, the column temperature was 40 °C, the flow rate was 1.5 mL min^{-1}, the injection volume was 20 μL and the measurement time was 20 min. Sodium acetate buffer (1000 mL) was composed of anhydrous sodium acetate 0.82 g (Wako, Osaka, Japan) dissolved in water, with 5 mL acetate (Kanto Chem, Tokyo, Japan) and diluted in a measuring flask to a total volume of 1000 mL with water. The mobile phase was composed of the ratio of sodium acetate buffer : acetonitrile (Sigma-Aldrich, Tokyo, Japan) in the ratio of 93 : 7 (vol/vol), and the solution was degassed for 5 min. The powder mixture was weighed using trace amounts from a powder dispenser R3 (Bio Dot, CA, USA) from 8 samples in each of the screw cans (4 points in the radial direction × 2 points in the vertical direction). These samples were diluted in a measuring flask to 10 mL using the mobile phase. The sample solution was confirmed to have dissolved sufficiently. Mean and SD were calculated as area per weight from each of the resulting peaks. Comparison of the variation in the concentration in the screw can was performed by calculating the coefficient of variation (CV) using eqn (10).

$$\mathrm{CV}\ (\%) = \frac{\mathrm{SD}}{\mathrm{mean}} \times 100 \tag{10}$$

3 Results and discussion

3.1 Numerical simulation by change in RAM parameters

The RAM method mixes the powder sample in a container by vertical vibration through the control of acceleration and frequency when the powder sample is thrown up. The powder sample is thrown up in small amounts from the surface by vibration, hence the amplitude of the container, vibration velocities and heights of the thrown up powder change with RAM parameters (acceleration and frequency), which are thus important factors. Therefore, a simulation of RAM parameters was performed in order to determine the appropriate acceleration and frequency conditions.

First, the amplitude was obtained by changing the frequency and acceleration based on eqn (5), as shown in Fig. 4a. The calculated amplitudes were small, hence the mixing efficiency was poor. Furthermore, the large amplitude requires a large scale mixing instrument; thus, the amplitude should be chosen for the scale of the instrument.

Next, vibration forces were applied to the particles, and vibration velocities were obtained by changing the frequency and acceleration based on eqn (6), as shown in Fig. 4b. The vibration velocity increased in proportion to the acceleration. In addition, F was proportional to the acceleration from eqn (11) based on Newton's second law of motion. When the velocities were higher, the collapse of the particles involved the collision energy of the particles.

$$F = ma \tag{11}$$

Finally, the calculated heights of the thrown up powder were obtained by changing the acceleration and frequency based on eqn (7), as shown in Fig. 4c. The acceleration and frequency could be predicted to be limited to within 50–130 G and 30–120 Hz based on the results shown in Fig. 4a and b, respectively. The heights of the thrown up powder were small, thus the mixing efficiency was poor. When the heights of the thrown up powder are larger, the powder collides head on and the particles collapse. Therefore, a suitable range for the heights of the thrown up powder were deduced between these limits. The results in Fig. 4 suggest the optimized operation parameters from the numerical simulations of amplitudes of the container, vibration velocities and heights of the thrown up powder by changing the RAM parameters (acceleration and frequency). From the above results, the optimum values of acceleration and

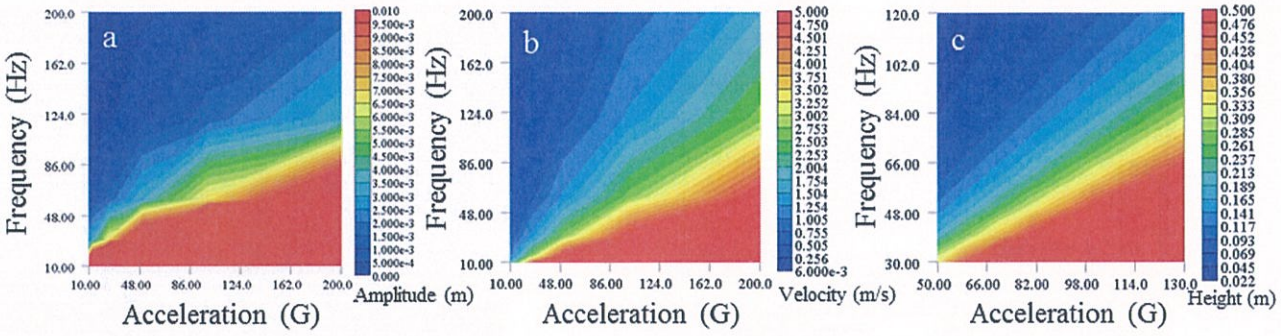

Fig. 4 Numerical simulations of (a) amplitudes of the container, (b) vibration velocities and (c) heights of the thrown up powder by changing the RAM parameters (acceleration and frequency).

frequency for the RAM method were 90–100 G and approximately 60 Hz, respectively. Herein, the frequency was set to around 60 Hz in order to transmit the force directly to the mixing plate.[20,21]

3.2 RAM parameters optimization

In the present study, mixing tests of fine powders and carrier lactose powders were performed by RAM and conventional mixing methods, as model formulation processes for the preparation of dry powder inhaler (DPI) preparations. When powders with significantly different particle sizes of about 10 and more than 100 times are mixed, it has been reported that they follow an ordered-mixture model (OM).[2,3] In general, powder flowability is proportional to the degree of OM formation. The angle of repose of the mixed powder samples by the RAM method was evaluated as powder flowability, and it reflected the optimization of the powder mixing conditions. In this section, MgO (fluidizing agent) was used as a model API in order to use powder flowability (angle of repose) as a specific powder characteristic of the mixed powder. Fig. 5 shows the effect of acceleration on angle of repose of the mixed powder sample (a mixture of MgO and lactose) by the RAM method. The angle of repose of the mixed powder was found to decrease with an increase in acceleration. This result indicates that powder flowability is improved by the RAM method. Additionally, the angle of repose decreased by extending the mixing time, and it was also confirmed that the powder flowability was improved. In the present powder formulation system, two types of powders were included, and the particle size diameters of MgO (3 μm) and lactose (more than 100 μm) differed by a factor of approximately 50. This result suggests the formation of an OM, because MgO was uniformly dispersed on the surface of the lactose. The experimental results of the angle of repose for the powder mixed using the RAM method (Fig. 3), and the results for the obtained RAM conditions (Fig. 4) by numerical simulations show a similar trend. All the results suggest that the optimal acceleration values for the RAM method are in the range of 90–100 G.

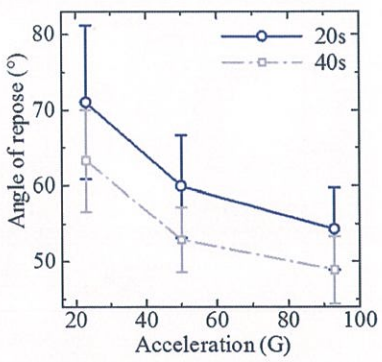

Fig. 5 Effect of acceleration on angle of repose of mixed powder by the resonant acoustic mixing method ($n = 30$, mean ± SD). Dark blue solid line is acoustic mixing for 20 s and light gray broken line is acoustic mixing for 40 s. SD, standard deviation.

3.3 Non-invasive verification of the mixing samples by RAM method

In order to confirm the non-destructive characteristics of the RAM method, mixing tests were carried out in the hard condition using lactose powder. The particle surface morphologies of samples LA1 and LA2 were not different in the rough condition before and after RAM in the SEM images (data not shown). Fig. 6 shows the frequency particle size distributions of LA1 and LA2, obtained from SEM images by imaging analysis. In addition, count median diameters (CMD) were also calculated from the data. The CMD of LA1 and LA2 were not different before and after mixing, and the particle size distributions also of both samples were not significantly different (one-way ANOVA, $p > 0.05$, $n = 3$) before and after mixing. These results indicate that mixing by the RAM method is highly non-invasive for powder particle samples, therefore, the method did not have a significant effect on the geometric structure of the powder sample particles. The powder was mixed in the container where the settings resulted in RAM in a weightless-state during the free-fall period, which was induced by throwing up of the sample. Mixing occurred without shear-stress in the weightless-state and is, therefore, able to prevent the disintegration of the particles.

3.4 Comparison study of the ordinary and the resonant acoustic mixing methods

As mentioned above, because the model formulation was used as a DPI for asthma medications, theophylline anhydrate was

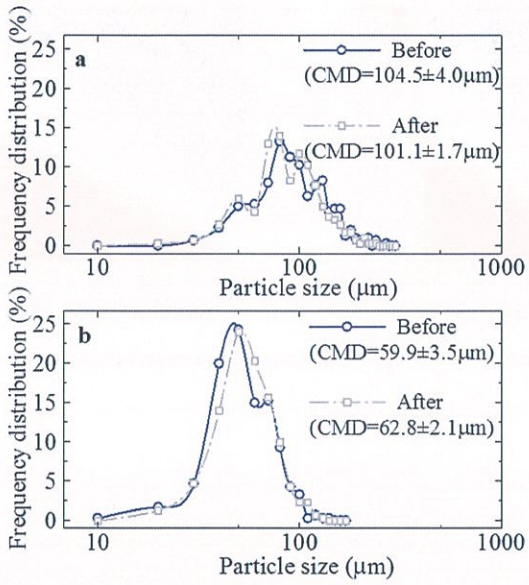

Fig. 6 Effect of mixing on particle size distribution of lactose by the conventional and resonant acoustic mixing methods. (a) Frequency distribution of LA1 and (b) frequency distribution of LA2, and the count median diameter (CMD ± SD) (n = 300). Dark blue solid line is before RAM and light gray broken line is after RAM. SD, standard deviation.

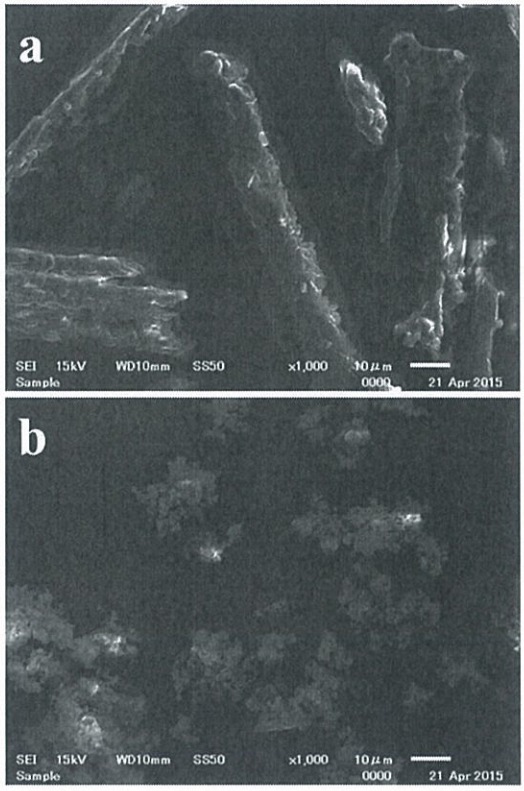

Fig. 7 SEM images of theophylline anhydride bulk powder. (a) Before pulverization (d = 24.4 μm), (b) after pulverization (d = 4.3 μm) (n = 3).

selected as the API. The mixing of the API and carrier lactose powders was performed by the RAM and modified V-shape blender methods, and then, content uniformity and powder flowability tests for the mixed powder samples were conducted to evaluate the mixed state of the formulation.

3.4.1 Micronization of the active pharmaceutical ingredient by a freeze wet milling method. Since DPI formulations require fine APIs, the APIs were pulverized by wet cryogenic grinding. Cryogenic milling is a simple method for grinding in an environment that enhances the brittleness of materials using the ultra-low temperature of liquid nitrogen. The sample can be separated spontaneously as a fine powder or as a liquid by returning the ground product to ambient temperature.

SEM images were recorded before and after milling of the API by cryogenic milling, which are shown in Fig. 7. The rectangular API crystals were confirmed to have been crushed to a state close to a sphere by this method. Furthermore, when the particle diameters before and after milling were measured based on specific surface area, the values were 24.4 μm and 4.3 μm, respectively. This result indicates that a sufficiently fine API for use in a DPI was obtained by the cryogenic grinding method.

3.4.2 Morphology of the distribution of active pharmaceutical ingredient in the mixed powders by the ordinary and resonant acoustic mixing methods. To confirm the distribution of the API in the formulation, the fine chemical structure of particles in the mixed powder was analyzed using ATR microscopy method.

Fig. 8 shows the FT-IR spectra of lactose monohydrate standard and API standard products by the ATR-IR method. The spectra were normalized in order to clarify their differences and to facilitate comparison. The specific absorption peak of lactose was observed at around 900 cm^{-1}, which is attributed to the symmetric stretching vibration of the C–O–C group. In contrast, the specific absorption of theophylline was observed at approximately 1700 cm^{-1} which is attributed to the C=O stretching vibration. Since both specific peaks did not overlap with each other, they could be used for chemical mapping by ATR-IR imaging.

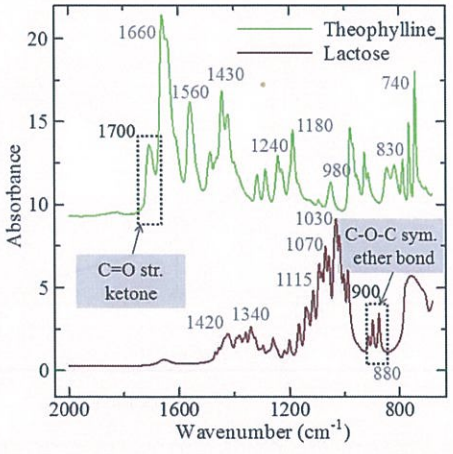

Fig. 8 Change of normalized infrared (IR) spectra of each component. Light green solid line is the active pharmaceutical ingredient (API) and dark red solid line is lactose.

The API and lactose powders were mixed for 0.5 h and 10 h (C1 and C2, respectively) at 30 rpm by the ordinary modified method, and the powders were mixed for 0.01 h and 0.03 h (C3 and C4, respectively) at 100 G and 60 Hz by the RAM method. Fig. 9a–d show the ATR-IR chemical mapping of the powder samples mixed by the ordinary and RAM methods. When particles of the API were mixed for the same mixing time, the RAM method was much more effective than the conventional method. After mixing for 0.5 h by conventional mixing (C1), aggregation of the secondary particles of the API (around 70 μm in diameter) was confirmed. The particles of the API in sample C2 mixed for 10 h were more uniformly mixed than the sample mixed for 0.5 h, however some aggregation of particles of around 25 μm in diameter was still observed. After mixing for only 0.01 h by the RAM method (C3), the aggregation of secondary particles of the API of 30 μm diameter was confirmed. However, in sample C4, which was mixed for 0.03 h, the particles of the API were uniformly dispersed by attachment to the lactose particle surfaces. When the RAM method was compared with the conventional method, the API was dispersed more finely using the RAM method. This suggests the higher capacity of the RAM method to disperse agglomerates than the conventional method. Therefore, further evaluation was carried out.

3.4.3 Powder flowability of mixed powders by ordinary and resonant acoustic mixing methods. The angle of repose of each sample was measured as an index of powder flowability and also the degree of powder mixing. Fig. 10 shows the effect of mixing conditions on the angle of repose of the mixed powder. The angle of repose of C2, C3, and C4 was significantly different from that of lactose powder ($p < 0.05$, $n = 3$, one-way ANOVA), but that of C1 was not different. This result suggests that the mixing conditions for C1 were not sufficient to produce a homogeneous mixed powder, and this is consistent with the results of the IR mapping. In general, it is well known that fine particles and coarse particles are not well mixed in standard mixers. In this study, the particle size of the fine particles and the carrier particles contained in the pharmaceutical

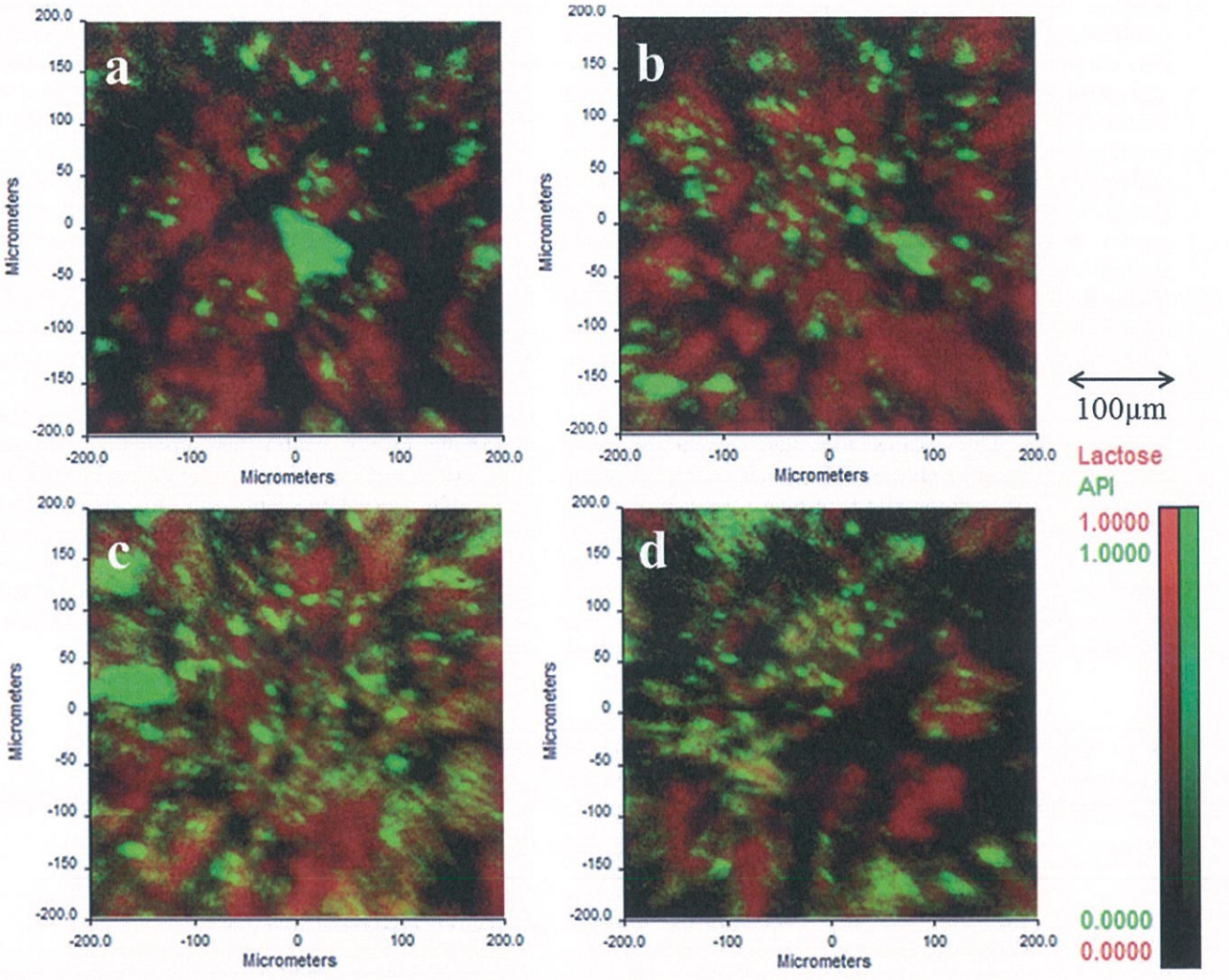

Fig. 9 Results of IR imaging by the attenuated total reflection (ATR) method. (a) C1 at 0.5 h and (b) C2 at 10 h with ordinary modify method, and (c) C3 at 0.01 h and (d) C4 at 0.03 h with the resonant acoustic mixing method. Light green areas are the API and dark red areas are lactose.

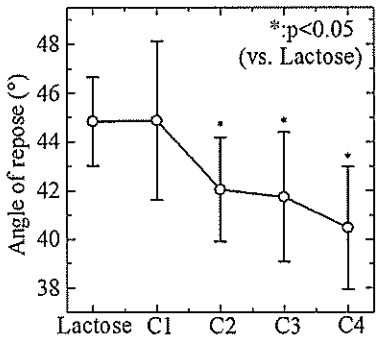

Fig. 10 Effect of mixing on angle of repose of the mixed powders by the ordinary and resonant acoustic mixing methods (n = 30, mean ± SD). SD, standard deviation.

formulation differed by a factor of more than 30. The cohesive force of a fine powder is strong, but the dispersion is not easy. In order to form an OM structure, fine particles of the API attached to the surface of the lactose carrier particles, therefore, it is necessary to have sufficient mixing shear stress that exceeds the cohesive force of the agglomerated particles. The mixing conditions in C1, C2, and C3 by the ordinary and RAM methods were not sufficient to generate an OM structure. In contrast, the aggregated powder was uniformly dispersed and mixed to form the OM structure in C4 conditions using the RAM method. It is considered that the formation of the OM led to the API being uniformly dispersed and adhered to the surface of lactose. It should be noted that, the tendency of RAM to have better powder flowability than the ordinary mixing method over approximately 10 hours was confirmed in spite of the very short mixing time. The particles collide at various locations within the container by the RAM method. Thus, the formation of an OM is dependent on the adhesion by intermolecular interactions between the API particles and the surface of the larger lactose particles.

3.4.4 Active pharmaceutical ingredient content uniformity of mixed powders by ordinary and resonant acoustic mixing methods. A drug uniformity test for pharmaceutical formulations was performed in accordance with the Japanese Pharmacopoeia XVI using HPLC. Table 1 shows the drug uniformity results of samples mixed using the conventional and RAM methods. The CV value of C1 was the largest and indicates non-uniformity. However, the CV values of C2, C3, and C4 were smaller than C1, and the results showed that the RAM method could obtain content uniformity approximately 900 times more rapidly compared with ordinary methods.

These results suggest that the formation of an OM is an ideal mixed state and could be realized in combination with non-invasiveness of the particles, good fluidity and uniform mixing. In this study, the RAM method could in an extremely short time produce uniform and efficient mixing of powders with different densities and particle systems.

Table 1 Comparative studies on the active pharmaceutical ingredient content uniformity between the ordinary and resonant acoustic mixing methods as a function of mixing time (n = 8)[a]

Run no.	Area/weight (mean)	SD	CV (%)
C1	6.29×10^{4}	1.35×10^{4}	21.4
C2	6.77×10^{4}	0.74×10^{4}	10.9
C3	6.62×10^{4}	0.54×10^{4}	8.2
C4	6.8×10^{4}	0.65×10^{4}	9.6

[a] CV, coefficient of variation; SD, standard deviation.

4 Conclusions

Experimental results and theoretical results for RAM conditions produced by numerical simulation showed similar tendencies, thus making it possible to predict optimal RAM parameters (acceleration and frequency). The RAM method was estimated to produce uniform powder mixing by utilizing free fall after throwing the powder upwards into the air. Therefore, with the RAM method it is possible to carry out mixing in a weightless state without depending on the density or mass of the sample. In other words, it is possible to prevent the disintegration of particles without causing stress to the particles. In addition, scale-up for industry is considered easy because the given acceleration is a constant value. The RAM method efficiently contributes to powder flowability through the formation of an OM in a very short period of time, and it can produce an ideal mixing state with ease. In addition, the RAM method offers simple operation in comparison with the ordinary method using a modified V-shape blender method, and it is expected to simplify pharmaceutical manufacturing facilities. Trace pharmaceutical powders were not mixed efficiently by the ordinary method, however, they were mixed efficiently by the RAM method. The RAM method also addresses various problems associated with the ordinary method. For example, there are some major problems in uniformity in the continuous production manufacturing processes of solid preparations, such as formulations consisting of pre-mixed excipients with a low drug content, which have particularly attracted attention in recent years. The RAM technique can be applied to mixing processes with low drug content formulations, and it will be important in the future. Therefore, RAM may be applicable to pharmaceutical manufacturing processes.

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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