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PAPER

Verification of the mixing processes of 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 related to dissolution and chemical stability in pharmaceutical manufacturing. Resonant acoustic® mixing (RAM) technology is a blending method, and it has reported that a unique mixing action for various samples. In this study, in order to apply the RAM method to pharmaceutical blending process, the optimization of the operating conditions of the RAM (acceleration and frequency) was conducted by numerical simulation. Powder mixing experiments were carried out using each of the RAM and the midified V-shaped mixing device as a powder material using theophylline powder and lactose or magnesium oxide and lactose. 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 were indicated that the optimum values for acceleration and frequency in RAM mixing were 90–100 G and around 60 Hz; and proved 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 to perform mixing by utilizing free-fall, possible by inducing a weightless state without depending on the density and mass of the sample. Therefore, RAM may be applicable to pharmaceutical manufacturing processes.

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 varied 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 mix 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 methods. 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 taking a long time 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 leaking out of active pharmaceutical ingredients (APIs) with high pharmacological activity.[14,15] Furthermore, because any ordinary mixing method is affected by the 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 real 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 were generated in various locations in the container, as shown in Figure 1.[17-21] In this case, the frequency

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was set to around 60 Hz in order to transmit the force directly to the mixing plate.[20,21] A vibration motion by plate's up-and-down movement occur after the container is fixed by the clamp and turn on the switch. That time, multiple mixing zone and bulk mixing flow occur in the container.[20,21] Because it is possible with this method to move powder samples 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. But, these reasons and mixing mechanism doesn't have made clear. Therefore, it is necessary to examine the optimal mixing parameter and the mixing mechanism of RAM in order to apply to blending process. Particular, many pharmaceutical samples are often extremely sensitive, and can be said to examine the things are important.

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

2 Experimental

2.1 Materials

The main paragraph text follows directly on here. Lactose monohydrate (DFE Pharma, Goch, Germany) Respitose® SV010 (d50: 105µm), Respitose® SV003 (d50: 60µm), Respitose® ML006 (d50: 17µm) and Lactohale® 100 (d50: 125 – 145µm) were used as the excipients. Magnesium oxide (MgO, 3µm, Wako, Osaka, Japan) was used as a lubricant. The API used was theophylline anhydride bulk powder (Shizuoka Caffeine, Shizuoka, Japan). All other chemicals were commercially available and of analytical grade. Milli-Q water was filtered using a Millipore system (Millipore, Billerica, MA, USA) and used for all samples.

2.2 Numerical simulation by change for RAM parameters

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

$$x = Acos\,(\omega t + \alpha) \tag{1}$$

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

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

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

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

Therefore, to calculate the amplitude by changing the frequency and acceleration from Eq. (5).[22]

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

Then, the powder was mixed by changing the frequency and acceleration of the RAM. The change in the force applied to the particles during mixing was predicted. Velocity, Eq. (6), was calculated by from Eq. (2) and Eq. (5). Here, 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 was calculated from Eq. (7) for the vertical upward projection.[22]

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

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 12.6g 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 set on the RAM, and powder samples were mixed at a constant frequency of 60Hz for 20s – 40s with a strength of 20G – 93G. In this case, the frequency was set to around 60Hz 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 23G – 20s, 61G – 20s, 93G – 20s, 23G – 40s, 61G – 40s, and 93G – 40s. It should be noted that 1.0G = 9.8m/s^2 in this report.

2.3.2 Flowability by angle of repose measurement

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

2.4 Non-invasive verification of the mixing samples by 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 screw stainless steel can (Shimizu Akira, Niigata, Japan, 150mL). The screw can set on the RAM (2.3.1), and it mixed at 100G – 150s at a frequency 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 (JOEL, Tokyo, Japan). Conditions were 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 coating order to reduce the charge-up phenomenon. In addition, Feret's diameter was measured from SEM images using image analysis software (Image-Pro Plus, Media Cybernetics, MD, USA). The particles were 100 counted for each sample. In this time, the overlapping particles were not counted. Measurements were performed 3 times.

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 nano - particles of API were obtained by cryogenic grinding in liquid nitrogen. API bulk powder (20g) was poured into a 500mL stainless steel pot with a cork-cap containing liquid nitrogen (-196°C) and 900g of zirconia beads (0.50mm in diameter). A tornado-blade (diameter 75mm) was rotated for 1h at a speed of 800rpm. 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. Morphology of the API after grinding was observed using a SEM. Conditions were magnification ×1000, high vacuum mode, but otherwise the conditions were as in 2.4.2.

2.5.2 Specific surface area measurement

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

$$S_w = \frac{S}{W} \quad (8)$$

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$$d = \frac{6}{\rho \cdot S_w} \quad (9)$$

2.5.3 Samples preparation by ordinary method using a modified V-shape blender method 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, total powder weight (40.0g) was placed in a screw stainless steel can (Shimizu Akira, Niigata, Japan, 150mL). As an ordinary mixing method using a V-blender, a modified V-shape blender method with a small volume (150mL) was made using a ball mill rotating gantry (AN2-10S, Nitto Kagaku, Nagoya, Japan) and fixed the screw can at angle 45°. Figure 3 shows the modified V-shape blender. After set on the modified V-shape blender, it mixed at 30rpm – 0.5h (C1) and 30rpm – 10h (C2). Also, the screw can set on the RAM (2.3.1) and it mixed at 100G - 0.01h (C3) and 100G-0.03h (C4) at a frequency of 60Hz.

2.5.4 Morphological observations of mixed powders

In order to confirm the uniform dispersion of the API, 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 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) software was used. Measurements used a single reflection ATR, resolution was 4.0 cm^{-1}, measurement range was 4000 – 400cm^{-1}, eight integrations were used, the ATR crystal was Diamond/KRS-5, incident angle was 45°, and the background was air as for FT-IR. IR spectra were normalized because this easy comparison clarified the difference between them. Measurements used ATR imaging, resolution was 8.0cm^{-1}, measurement range was 4000 – 680cm^{-1}, a single integration was used, the ATR crystal was Ge, pixel size was 1.56µm × 1.56µm, measurement area was 400µm × 400µm, 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 used 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 by a high-performance liquid chromatography (HPLC). A LC-10vp series HPLC (Shimadzu, Kyoto, Japan), analysis software CLASS-VP 6.14SP1 (Shimadzu, Kyoto, Japan) and an Inertsil® ODS – 3 5µm 4.6 × 250 mm column (GL Sciences, Tokyo, Japan) were used. The detector was an ultraviolet absorption spectrophotometer (measurement wavelength: 228 nm), the quantitative method used absolute calibration, the column temperature was 40°C, flow rate 1.5mL/min, injection volume was 20µL and measurement time 20min. Sodium acetate buffer (1000mL) was composed of anhydrous sodium acetate 0.82g (Wako, Osaka, Japan) dissolved in water, with 5mL acetate (Kanto Chem, Tokyo, Japan) and diluted in a measuring flask to a total volume of 1000mL with water. Mobile phase was composed of sodium acetate buffer : acetonitrile (Sigma-Aldrich, Tokyo, Japan) at 93 : 7 (vol : vol), and the solution was degassed for 5min. 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 measuring flask to 10mL 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 Eq. (10).

$$CV\ (\%) = \frac{SD}{Mean} \times 100 \quad (10)$$

3 Results and discussion

3.1 Numerical simulation by change for 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. Because throwing up of the powder sample occurs in small amounts from the surface by vibration, the amplitude of the container, vibration velocities and heights of the throwing up powder by change for RAM parameters (acceleration and frequency) are important factors. Therefore, a simulation of RAM parameters was performed in order to determine the appropriate acceleration and frequency conditions.

First, the amplitude obtained by changing the frequency and acceleration based on Eq. (5). It is shown in Figure 4a. The calculated amplitudes were smaller; the mixing efficiency was poor. Also, the calculated amplitudes were lager; the instrument become large and become unsuitable a laboratory scale.

Next, in order to the vibration forces were applied to the particles, the vibration velocities obtained by changing the frequency and acceleration based on Eq. (6). It is shown in Figure 4b. The vibration velocity increased in proportion to the

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acceleration. In addition, F was proportional to acceleration from Eq. (11) based on Newton's second law of motion. When the velocities were lager; the collapse of the particles involved the collision energy of the particles.

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

Finally, the calculation result of the heights of the throwing up powder obtained by changing the acceleration and frequency based on Eq. (7). It is shown in Figure 4c. The acceleration and frequency could be predicted to be limited to within 50G – 130G and 30Hz – 120Hz based on the results are shown in Figure 4a and 4b, respectively. The heights of the throwing up powder were smaller; the mixing efficiency was poor. Also, the heights of the throwing up powder were lager; hit on the head and the collapse of the particles. Therefore, a suitable range for the heights of the throwing up powder were between these limits. The results in Figures 4 suggested that the optimized operation parameters from the numerical simulations of amplitudes of the container, vibration velocities and heights of the throwing up powder by change for RAM parameters (acceleration and frequency). From the above results, the optimum values of acceleration and frequency for the RAM method were 90–100 G and around 60 Hz, respectively. In this time, the frequency was set to around 60Hz in order to transmit the force directly to the mixing plate.[20,21]

3.2 RAM parameters optimization

In the present study, the 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 followed 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. Figure 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 decreased with increasing acceleration. The result indicated that powder flowability was 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 kinds of powders were included, and the particle size diameters of MgO (3μm) and lactose (more than 100μm) differed by a factor of approximately 50 times. This result suggested the formation of an OM, because MgO was uniformly dispersed on the surface of the lactose. Angle of repose experimental results for the powder mixed using the RAM method (Figure 3) and the results for the obtained RAM conditions (Figures 4) by numerical simulations showed a similar trend. All results suggested that optimal acceleration values for the RAM method were 90–100G.

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 at the hard condition using lactose powder. The particle surface morphologies of samples LA1 and LA2 were not different in the rough between before and after RAM in SEM images (data not shown). Figures 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 difference between before and after mixing, the particle size distributions of both samples were not significantly different (one-way ANOVA, $p>0.05$, $n=3$) between before and after mixing. The results indicated that mixing by the RAM method was highly non-invasive for the powder particle sample; 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 induced by throwing up of the sample. The 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 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

Because DPI formulations require fine APIs, 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 method 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. They are shown Figures 7. The rectangular API crystals could be confirmed to have been crushed to a state close to a sphere by this method. Furthermore,

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when the particle diameters before and after milling were measured based on specific surface area, the values were 24.4μm and 4.3μm, respectively. The result indicated that a sufficiently fine API for use in a DPI was obtained by the cryogenic grinding method.

3.4.2 Morphology observation 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 the microscopy ATR method.

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

The API and lactose powders were mixed for 0.5h and 10h (C1 and C2, respectively) at 30rpm by ordinary modify method, and the powders were mixed for 0.01h and 0.03h (C3 and C4, respectively) at 100G, 60Hz by the RAM method. Figures 9a – 9d shows ATR-IR chemical mapping of the powder samples mixed by the ordinary and RAM methods. When particles of the API were mixed for same mixing time, the RAM method was much more effective than the conventional method. After mixing for 0.5h by the conventional mixing (C1), aggregation of secondary particles of the API (around 70 μm in diameter) was confirmed. The particles of the API in sample C2 mixed for 10h were more uniformly mixed than the sample mixed for0.5h, but some aggregation of particles of around 25μm in diameter was still observed. After mixing for only 0.01h by the RAM method (C3), aggregation secondary particles of the API of 30μm diameter was confirmed. However, in sample C4 mixed for 0.03h, 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 suggested a 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. Figure 10 shows the effect of mixing condition on the angle of repose of the mixed powder. The angle of repose of C2, C3, and C4 were significantly different from that of lactose powder ($p<0.05$, $n=3$, one-way ANOVA), but that of C1 was not different. The result suggested that the mixing conditions for C1 were not sufficient to produce a homogeneous mixed powder, and this was 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 formulation differed by a factor of more than 30 times. The cohesive force of a fine powder is strong, and the dispersion of fine powders is not easy. In order to form an OM structure, fine particles of the API became attached to the surface of lactose carrier particles; therefore, it is necessary to have sufficient mixing shear stress that exceeded 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 was considered the formation of an OM led to the API being uniformly dispersed and adhered to the surface of lactose. Of note, the tendency of RAM that the powder flowability was better 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 by the conventional and RAM methods. The CV value of C1 was the largest and indicated non-uniformity. However, the CV values of C2, C3, and C4 were smaller C1, and the results showed that the RAM method could obtain content uniformity approximately 900 times more rapidly compared with ordinary methods.

These results suggested that the formation of an OM was 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.

Conclusions

Experimental results and theoretical results for RAM conditions produced by numerical simulation showed similar tendencies, 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,

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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 the particles without causing stress to the particles. In addition, scale-up for industry is considered easy because the given acceleration was a constant value. The RAM method efficiently contributes to powder flowability through the formation of OM in a very short period of time, and it could produce an ideal mixing state with ease. In addition, the RAM method offers simple operation in comparison with 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 ordinary method; however, these were mixed efficiently by the RAM method. The RAM method also addressed various problems associated with the ordinary method. As an example, there are some major problems in uniformity in continuous production manufacturing processes of solid preparations, such as formulations consisting of pre-mixed excipients with low drug content, which are particularly attracting 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.

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Declaration of interests

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

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Figures captions

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

Figure 2. The modified gradient method instrument in order to measure small amount of powder**.**

Figure 3. The modified V-shape blender method as the ordinary mixing method.

Figure 4. Numerical simulations of (a) amplitudes of the container, (b) vibration velocities and (c) heights of the throwing up powder by change for RAM parameters (acceleration and frequency).

Figure 5. Effect of acceleration on angle of repose of mixed powder by the resonant acoustic mixing methods. (n=30, mean ± SD). Dark blue solid lines are acoustic mixing for 20s and light gray broken lines are acoustic mixing for 40s. SD, Standard Deviation.

Figure 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 lines are before RAM and light gray broken lines are after RAM. SD, Standard Deviation.

Figure 7. SEM images of the theophylline anhydride bulk powder. (a) Before pulverizing (d=24.4μm), (b) After pulverizing (d=4.3μm) (n=3).

Figure 8. Change of normalized infrared (IR) spectra of each component. Light green solid lines are the active pharmaceutical ingredient (API); dark red solid lines are lactose.

Figure 9 The results of IR imaging by the attenuated total reflection (ATR) method. (a) C1 at 0.5h and (b) C2 at 10h with ordinary modify method, c) C3 at 0.01h and (b4) C4 at 0.03h with the Resonant acoustic mixing method. Light green areas are the API and dark red areas are lactose.

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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).

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

CV, coefficient of variation; SD, standard deviation.

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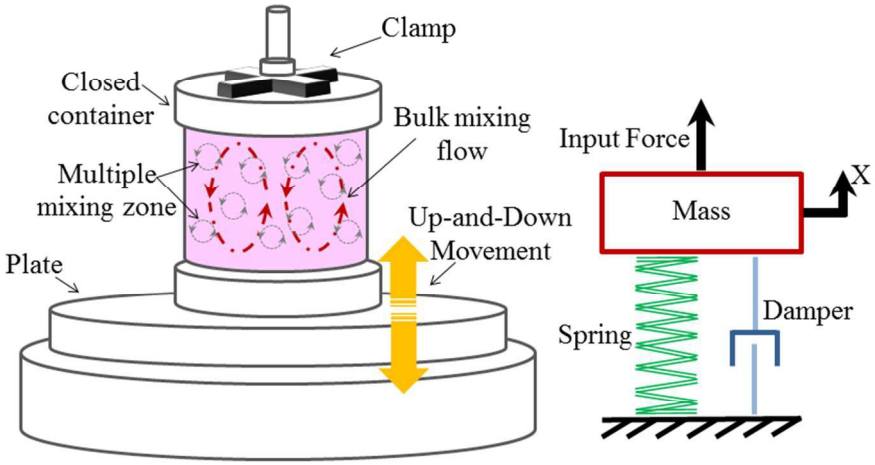

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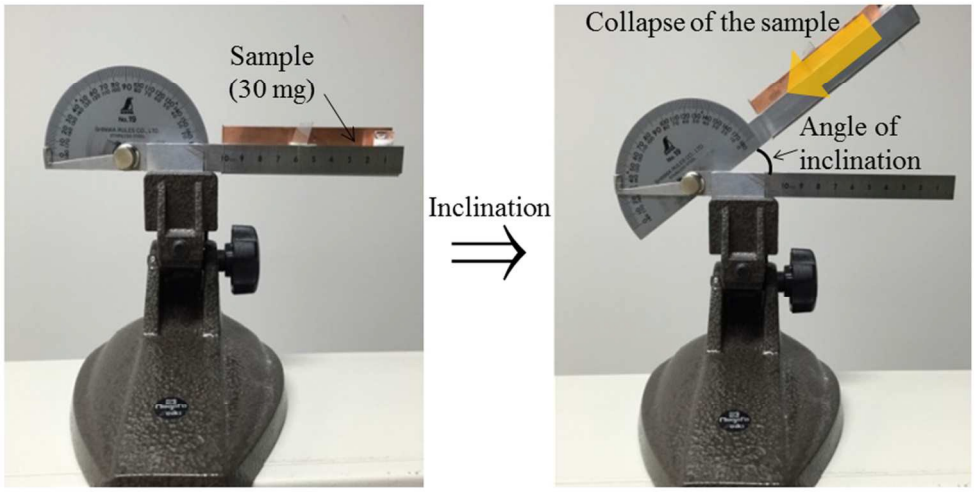

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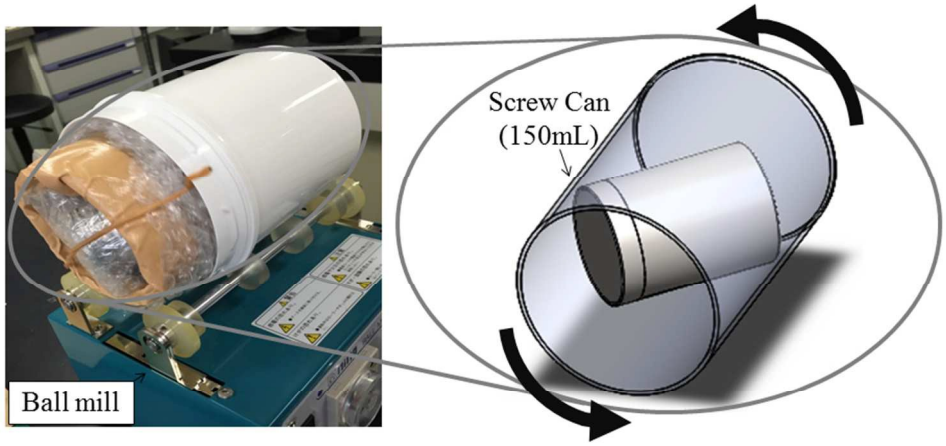

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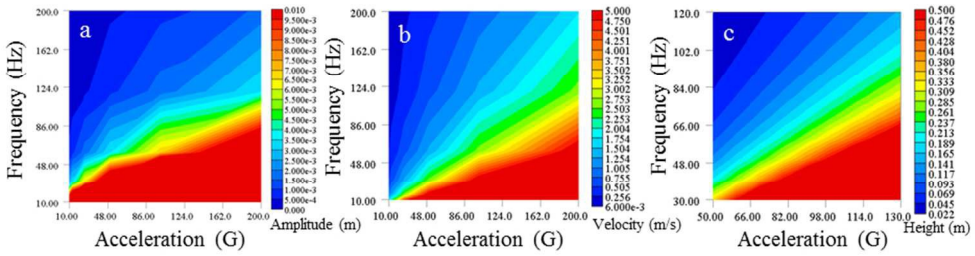

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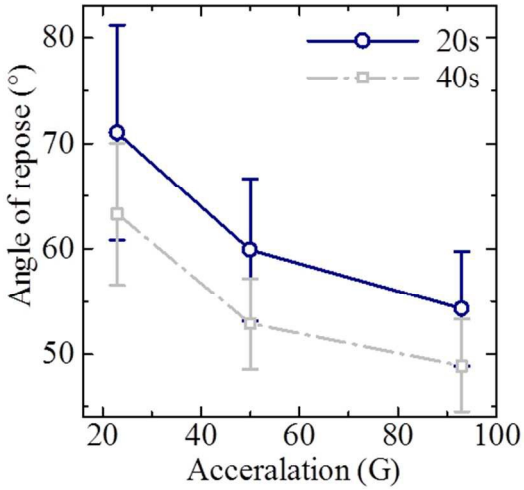

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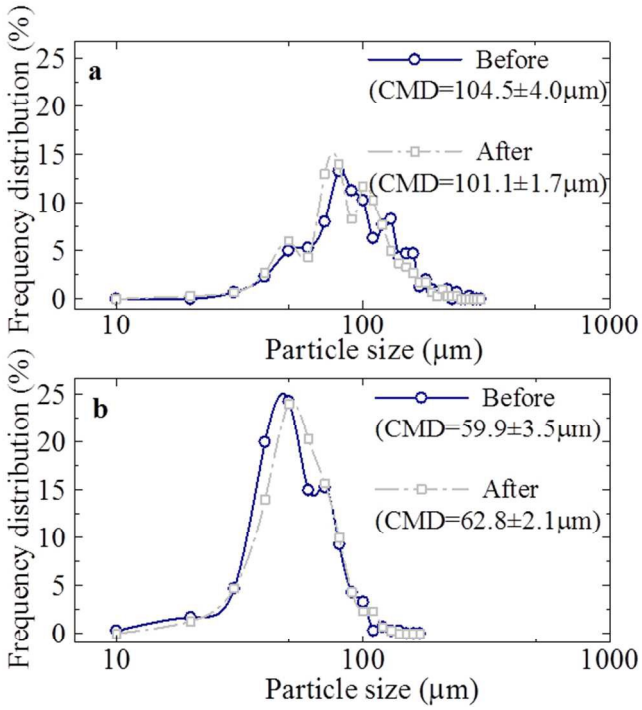

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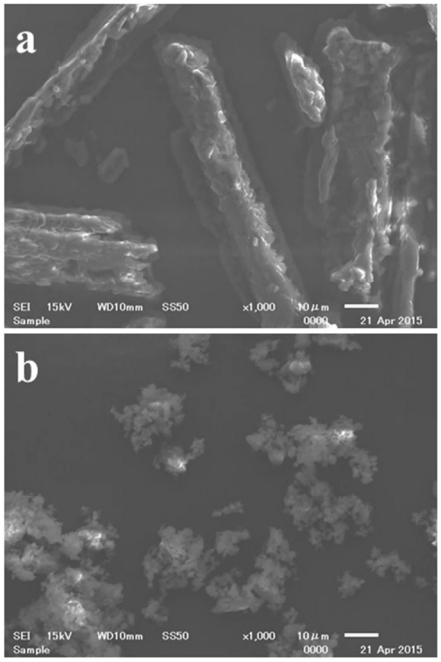

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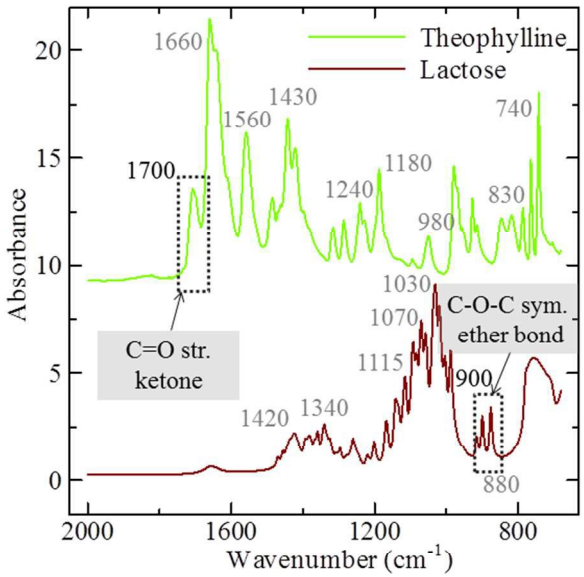

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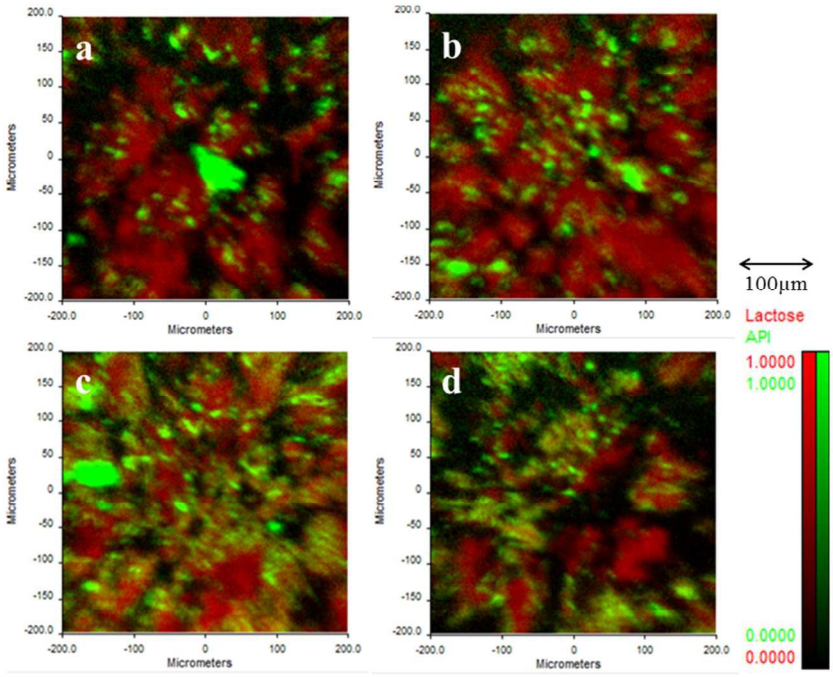

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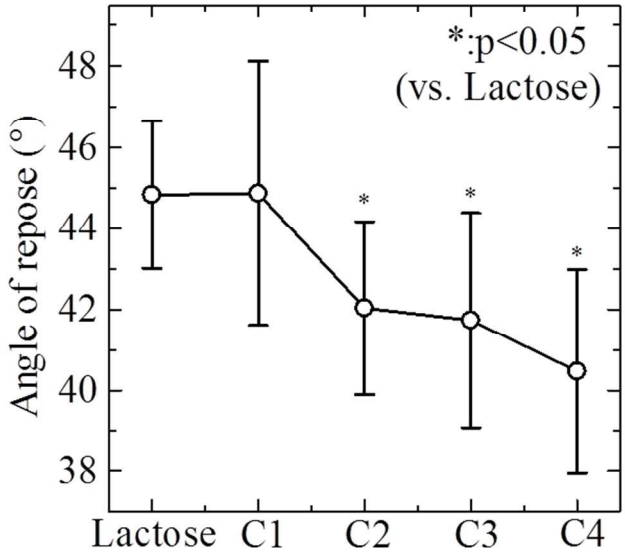

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