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# Novel technology to prepare oral formulations for preclinical safety studies

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**Abstract**

A novel method to prepare oral formulations, normally suspended dosage form, for preclinical safety studies in animals has been developed using a rotation/revolution mixer. Small hard balls made of zirconia were added to the mixing process to evaluate effectiveness in making a high quality suspension. The driving with balls loaded in the cylindrical container (vessel) of the mixer was quite efficient in dispersing and milling the particles of the active pharmaceutical ingredient (API) in an aqueous medium. The API powder and a small amount of oral aqueous medium (vehicle) were successfully mixed by the spinning motion of the balls in the vessel as though the paste-like suspension was kneaded with a mortar and pestle. It was found that the milled suspension with the mean size of 10–20 μm could be prepared, in addition finer milling of less than 10 μm could be achieved by selecting the material of vessel. Optimum driving conditions including mixing time, size and quantity of balls, and the standard operational procedure was established using compounds varying in physicochemical properties. The particle size and quantitative analysis by HPLC showed that the resultant suspension was well-milled and highly homogeneous with the nearly intended concentration of API. The proposed method established by this experiment could be applied to the actual safety studies in the real preparation scale of oral suspension.


*Keywords:* Suspension; Preclinical safety; Rotation/revolution mixer; Zirconia balls; Milling; Oral formulation

## 1. Introduction

In the past decade, drug discovery has gone through significant changes and shifts in paradigm with the pharmaceutical industry, which has been both exciting and challenging. Through the utilization of high throughput screening (HTS) a vast number of "leads" have been identified on the basis of in vitro potency and selectivity (Lipinski, 2000). Such HTS paradigms push to provide promising compounds, so-called "candidates", with speed, which are further evaluated in vivo against a targeted pharmacokinetic and safety profile (Bajpai and Adkison, 2000). These in vivo studies usually require each candidate to be rapidly formulated for parenteral administration such as intravenous, subcutaneous or intraperitoneal (Bittner and Mountfield, 2002a; Lee et al., 2003; Strickley, 2004).

In contrast with the state-of-the-art HTS technology, oral formulation, which is usually dosage form suspended the compound in aqueous medium, is manually prepared by the conventional method using a mortar and pestle because this method is highly flexible for various situations of dosing such as restricted amounts and availability of compounds, and a wide range of concentrations on a small preparation scale. As progressed to the preclinical stage, the highest doses are increased to typically 100-fold $ED_{50}$ (50% efficacious dose) or to an FDA-recommended maximum of 2 g/kg in the case where the compound does not exhibit adverse effects at preclinical safety studies before nominating a compound to phase I studies in humans (Neervannan, 2006). In addition, the dosing period is prolonged up to 28 days and the safety studies in non-rodent animals such as dogs are also required from a regulatory perspective. Although such situations significantly increase the manufacturing scale and frequency at the late preclinical stage, e.g. 60 g of API, 2000 mL of suspension, to our knowledge the conventional preparation method with a mortar/pestle is still

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applied to prepare oral suspensions. However, the manufacturing operation with a mortar/pestle is very time-consuming and the quality of the suspension, such as suspended particle size distribution, is operator-dependent. In addition, a variety of compound morphology and polymorph from batch to batch is likely to cause heterogeneous drug distribution in dosage and, hence variable dosing (Bittner and Mountfield, 2002b).

In this paper, the novel preparation method was developed with the unique mixer in order to mitigate these disadvantages of the conventional mortar/pestle method. The operational procedure with the mixer was improved to expand the use of the mixer to various types of compounds with physicochemical challenges. The quality of the prepared suspension was evaluated from morphological and homogeneitical perspectives. Further, some preparation conditions were optimized and proposed to apply to the actual safety studies at the real preparation scale of oral suspension.

## 2. Materials and methods

### 2.1. Manufacturing instruments

The rotation/revolution mixer (AR-250, Thinky Co. Ltd., Tokyo, Japan) was used to prepare oral suspension of the compounds. The 150 mL capacity vessel (Thinky, Japan) made of high-density polyethylene (HDPE) was primarily used in the present experiment. The vessel made of stainless steel (SS) was used as needed. Zirconia (zirconium oxide) balls with 1, 3, and 5 mm in diameter (YTZ-1, -3, -5) were purchased from Nikkato Co. Ltd. (Osaka, Japan).

### 2.2. Chemicals

Chemically synthesized compounds A–D as "candidates" of the active pharmaceutical ingredient (API) are proprietary compounds of Pfizer Global Research and Development (Nagoya, Japan). Phenytoin was purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). The aqueous solubility and particle size distribution of each compound is listed in Table 1. Methylcellulose (Metolose SM-4000) and Tween 80 (polysorbate 80) were purchased from Shin-Etsu Co., Ltd. (Tokyo, Japan) and Nikko Chemicals Co., Ltd. (Tokyo, Japan), respectively.

Table 1
Physical attributes of compounds applied in the current experiments

| Compound | Melting point[a] (°C) | Solubility in water (μg/mL) | Particle size (μm)[b] | |
|---|---|---|---|---|
| | | | $D$[4,3][c] | $D_{10}$, $D_{50}$, $D_{90}$[d] |
| Phenytoin | 296 | 22 | 40 | 19, 34, 64 |
| A | 186 | 120 | 147 | 30, 134, 279 |
| B | 191 | 2.0 | 47 | 8, 32, 100 |
| C | 242 | 1.7 | 72 | 24, 70, 114 |
| D | 158 | 440 | 11 | 1, 8, 25 |

[a] Endothermic onset temperature.
[b] Measured by Mastersizer 2000, Malvern.
[c] Volume moment mean diameter.
[d] Diameters at the 10%, 50% and 90% of the population distribution.

### 2.3. Preparation of oral suspension

The wet dispersing and milling was executed by spinning zirconia balls within the vessel. Phenytoin was used as an initial approach to roughly set up the driving condition with the mixer. Next, compounds A–D were applied to optimize the size and quantity of zirconia balls loaded and to determine operational process fit for actual manufacturing of the dosage formulations in safety studies. The representative formulations and operational conditions to prepare the oral suspensions are tabulated in Table 2. The total amount of compound to be formulated into oral suspension was weighed into the vessel of the mixer. The various size/quantity of zirconia balls were put into the vessel and the appropriate volume of aqueous medium (vehicle) was added. Then, the contents of the vessel were mixed by rotating and simultaneously revolving the vessel in the mixer at the fixed driving condition as follows: mixing mode (rotation: 800 rpm, revolution: 2000 rpm) for 1 min and de-foaming mode (rotation: 60 rpm, revolution: 2200 rpm) for 30 s. The additional mixing was repeated until solid material was fully wet and evenly dispersed. Then, the remainder of the vehicle was poured into the suspension using a pipette to dilute to the target concentration. A 0.5% methylcellulose aqueous solution (0.5% MC) was used as a standard oral vehicle. When the compound was too hydrophobic to be evenly dispersed, 0.1% Tween 80 was added to promote wetting to aqueous vehicle. As a reference of grinding performance, 150 mg of compound A was ground and dispersed into 2.5 mL of 0.5% MC to prepare suspension by a conventional method with a mortar/pestle.

### 2.4. Morphology and particle size distribution (PSD)

The morphology and particle size of the crystals were visually observed by polarized light microscopy (PLM) using a BX50 microscope (Olympus, Tokyo, Japan) and DS-U1 digital camera (Nikon, Tokyo, Japan). The particle size distributions of the original crystals dispersed in 0.04% polyoxyalkylene alkylether (Naroacty N-95, Sanyo Chemicals, Kyoto, Japan) aqueous solution and the milled particles in the prepared suspension were measured by a laser diffractometer (Mastersizer, 2000, Malvern Instruments, England) with a small-volume dispersing unit (Hydro 2000 μP, Malvern Instruments). The equivalent volume moment mean diameter $D$[4,3], and the diameters at the 10%, 50%, and 90% of the population distribution ($D_{10}$, $D_{50}$, $D_{90}$, respectively) were represented as mean particle size and size distribution. In some cases, the size distribution was additionally measured by an image analyzer in flowing cell (FPIA-3000S, Sysmex, Kobe, Japan) to check the consistency of measurement data. The aliquot of aqueous suspension was dispersed in water and photographs were taken storoboscopically while laminarly flowing the particles through the thin-layered cell. The mean diameter of the circles with equal projected area to particles (Heywood diameter), and the diameters at the 10%, 50%, and 90% of the population distribution ($D_{10}$, $D_{50}$, $D_{90}$, respectively) were also represented.

Table 2
Formulations and operational conditions for preparation of oral suspension by the rotation/revolution mixer

| Trial # | Compound | Nominal concentration (mg/mL) | Weight of API[a] (mg) | Volume of vehicle (mL)[b] | Balls | Vessel[c] | Addition steps of vehicle (mL)[d] |
|---|---|---|---|---|---|---|---|
| 1 | A | 10 | 100 | 9.923 | No balls | HDPE, 150 mL | 0.3 → 1 → 8.623 |
| 2 | A | 10 | 100 | 9.923 | 5 mm Ø × 3 balls[e] | HDPE, 150 mL | 0.3 → 1 → 8.623 |
| 3 | A | 10 | 100 | 9.923 | 3 mm Ø × 15 balls[e] | HDPE, 150 mL | 0.3 → 1 → 8.623 |
| 4 | A | 10 | 100 | 9.923 | 1 mm Ø × 380 balls[e] | HDPE, 150 mL | 0.3 → 1 → 8.623 |
| 5 | A | 60 | 600 | 9.538 | 3 mm Ø × 15 balls | HDPE, 150 mL | 0.5 → 0.5 → 0.5 → 8.038 |
| 6 | A | 6 | 1,800 | 299 | 3 mm Ø × 30 balls | HDPE, 150 mL | 1.44 → 0.36 → 3 |
| 7 | A | 6 | 2,220 | 368 | 5 mm Ø × 3 balls | HDPE, 150 mL | 1.8 → 0.2 → 0.2 → 5 |
| 8 | A | 60 | 22,200 | 353 | 5 mm Ø × 9 balls | HDPE, 150 mL | 14 → 0 → 4 → 10 |
| 9 | B | 60 | 600 | 9.538 | 3 mm Ø × 15 balls | HDPE, 150 mL | 0.6 → 0.3 → 0.3 → 8.338 |
| 10 | B | 60 | 600 | 9.538 | 3 mm Ø × 15 balls | HDPE, 150 mL | 0.6 → 0.2 → 0.2 → 0.2 → 0.2 → 1[f] → 7.138 |
| 11 | B | 60 | 600 | 9.538 | 3 mm Ø × 15 balls | SS, 150 mL | 0.6 → 0.3 → 0.3 → 8.338 |
| 12 | C | 10 | 730 | 73 | 3 mm Ø × 15 balls | HDPE, 150 mL | 0.7 → 0.35 → 10 |
| 13 | C | 30 | 2,190 | 71.32 | 5 mm Ø × 3 balls | HDPE, 150 mL | 1.6 → 0.4 → 0.4 → 15 |
| 14 | C | 100 | 7,300 | 67.38 | 5 mm Ø × 6 balls | HDPE, 150 mL | 4.4 → 1.3 → 1.3 → 10 |
| 15 | D | 10 | 20,100 | 1985 | 5 mm Ø × 9 balls | HDPE, 150 mL | 12 → 4 → 4 |

[a] API: active pharmaceutical ingredient.
[b] The density of compound is assumed to be 1300 mg/mL.
[c] HDPE: high density polyethylene, SS: stainless steel.
[d] 0.3 → 1 → 8.263: add 0.3 mL of vehicle into the vessel and drive the mixer at fixed driving condition (mixing mode for 1 min and de-foaming mode for 30 s). Next, add 1 mL of vehicle and drive the mixer at same condition. Then, add remainder of preset volume of vehicle (8.263 mL) and drive the mixer at same condition.
[e] The total weight of balls was unified to approximate 1.2 g.
[f] At Trial #10, the driving time was prolonged to "mixing mode for 5 min and de-foaming mode for 30 s" only after adding 1 mL of vehicle.

### 2.5. Homogeneity of suspension

The contents of the finished suspension were assayed to assess its homogeneity. The suspension prepared at the desired concentration was stirred to mix well without foaming. When stopped mixing, and having been rest for at least 10 s, each three 200 μL aliquots of the suspension were individually withdrawn from the upper, middle and lower parts of the whole suspension to measure the concentration of the compound. Nine samples were separately diluted in acetonitril/water (50/50, v/v) to the appropriate concentration and assayed by the HPLC system (Alliance 2695 with PDA setector, Waters) with each condition of column and mobile phase established for each compound. The homogeneity of the suspension, which is composed of concentration and content uniformity, was represented by the mean concentration and relative standard deviation (%) of upper, middle and lower content.

## 3. Results and discussion

### 3.1. Concept of novel application of a rotation/revolution mixer

The rotation/revolution mixer is a new type of one-pot mixer without having any propeller-type agitators. The cylinder-shaped material container with a lid (vessel) rotates while simultaneously revolving in the mixer at a 400-gravity acceleration, which results in the highest-grade mixing possible with no air bubbles. It is used in a number of fields and industries such as cosmetics, electronics, semiconductors, adhesive and sealants, film, plastics, chemicals, foods, pharmaceuticals and paints and coatings to prepare solder paste, ointment, and epoxy resin to name a few (Miyamatsu et al., 2004; Ishii, 2005). The preliminary manufacturing studies using phenytoin, which is freely available on the market, indicated that the un-wet aggregated mass of powder, called clumping, tends to be easily produced and disturbs the homogeneous dispersion especially when manufacturing on a large scale over several hundred milligrams of compound. The shortage of power for mixing required to mix the excessive amount of powder and to prepare thick suspension has limited the application of the mixer in many cases so far.

In order to expand the applications of the mixer in preclinical studies, it was attempted to execute the wet kneading and/or wet milling process while driving the mixer. In fact, the loading of hard small balls into the vessel of the mixer was suggested to aid and enhance the power of dispersion. The balls made of zirconia, zirconium oxide, were adopted in the current mixing procedure because they are readily available on the market and have an excellent frictional wear-resistant property. They have been frequently used in dry milling due to their heavy density, low price and variety of sizes available. The preliminary manufacturing experiments at a scale of 1–40 g loading of phenytoin were carried out to roughly set up the driving conditions of the mixer in combination with zirconia balls. As a result, it was found that the mixer should be run to mimic the kneading process of the conventional method with a mortar/pestle. That is, the powder of a compound was mixed with a part of vehicle and balls as if to knead the paste in the vessel at the initial mixing step. This kneading process was also found to be significantly effective in reducing the particle size as well as deaggregating the un-wet mass to provide the homo-

Table 3
Particle size distribution of original API powder and suspensions of compound A prepared with a mortar/pestle

| Sample | Grinding time | Particle size (μm)[a] | |
|---|---|---|---|
| | | $D$[4,3][b] | $D_{10}$, $D_{50}$, $D_{90}$[c] |
| Original API powder | – | 147 | 30, 134, 279 |
| Suspension (60 mg/mL) | 1 min | 55 | 8, 41, 119 |
| | 5 min | 17 | 2, 9, 36 |

[a] Measured with Mastersizer 2000, Malvern.
[b] $D$[4,3]: volume moment mean diameter.
[c] Diameters at the 10%, 50% and 90% of the population distribution.

geneous suspension. The preparation process is similar to the well-known high-shear media milling technology which provides aqueous dispersions consisting of nanometer-sized drug crystals (Merisko-Liversidge et al., 1996; Merisko-Liversidge et al., 2003; Wu et al., 2004; Jinno et al., 2006). However, the novel method using a rotation/revolution mixer with zirconia balls focuses on simple preparation of normal suspensions consisting of micrometer-sized crystals to switch over from the manually operational method with a mortar/pestle. In addition, this method is developed to support animal studies at the discovery and preclinical stages. It does not cover the development of the commercial dosage form. The experimental data using the proprietary compounds from Pfizer are shown in the following section.

### 3.2. Preparation at a smaller manufacturing scale than 1 g loading of the compound

As the reference of mixing and grinding performance, the suspension (60 mg/mL concentration) of compound A, which has large crystals as shown in Table 1, was prepared by the conventional preparation method with a mortar/pestle. One batch was ground for 1 min, and another for 5 min, respectively. It was found that there was difference in particle size distribution (PSD) between both batches as shown in Table 3. The long grinding time resulted in an effective decrease in the particle size. This result suggested that the conventional method with a mortar/pestle could cause uneven quality from the particle size perspective. Han et al. reported that poor bioavailability and erratic plasma concentration were observed during clinical studies after oral administration of extemporaneously prepared tacrolimus suspensions having 60–70 μm in mean particle size (Han et al., 2006). Therefore, PSD ($D_{10}$, $D_{50}$, $D_{90}$ = 2, 9, 36 μm) with sufficient grinding is set as target values for the preparation by the rotation/revolution mixer especially considering the oral administration of poorly water-soluble compounds.

The size and quantity of the balls were changed at 100 mg/10 mL loading scale in Trials #1–4 using compound A to optimize the driving condition. According to the results of preliminary experiments using phenytoin, the preset small volume of vehicle (0.3 mL) was added and the mixer was driven to promote even dispersion of powder. Then, 1 mL of vehicle was added and the mixer was driven again. After the sufficient dispersed suspension was visually observed, the remainder of the

Table 4
Particle size distribution and concentration/homogeneity of suspensions of compound A prepared with the rotation/revolution mixer

| Trial # | Nominal concentration (mg/mL) | Preparation scale (API, mg/suspension, mL) | Balls | Particle size (μm) | | | | | Assayed conc. (mg/mL) | | | Mean (mg/mL) | R.S.D. (%)[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Laser diffraction[b] | | Image analysis[c] | | | Upper | Middle | Lower | | |
| | | | | $D$[4,3][d] | $D_{10}$, $D_{50}$, $D_{90}$[e] | Heywood[f] | $D_{10}$, $D_{50}$, $D_{90}$[e] | | | | | | |
| – | Original API powder | | | 147 | 30, 134, 279 | ODL[g] | ODL | | – | – | – | – | – |
| 1 | 10 | 100/10 | No balls | 155 | 26, 140, 300 | ODL | | ODL | 2.46 | 3.61 | 17.98 | 8.02 | 107.9 |
| 2 | 10 | 100/10 | 5 mm Ø × 3 balls[h] | 30 | 3, 17, 75 | 50 | | 14, 52, 81 | 9.44 | 9.93 | 10.29 | 9.89 | 4.35 |
| 3 | 10 | 100/10 | 3 mm Ø × 15 balls[h] | 14 | 2, 9, 28 | 13 | | 4, 11, 29 | 9.49 | 9.84 | 9.82 | 9.72 | 2.00 |
| 4 | 10 | 100/10 | 1 mm Ø × 380 balls[h] | 116 | 4, 102, 264 | 81 | | 16, 85, 117 | 8.59 | 9.60 | 10.75 | 9.65 | 11.22 |
| 5 | 60 | 600/10 | 3 mm Ø × 15 balls | 10 | 1, 7, 21 | 13 | | 4, 10, 25 | 58.72 | 58.68 | 59.34 | 58.92 | 0.63 |

[a] R.S.D.: relative standard deviation.
[b] Measured with Mastersizer 2000, Malvern.
[c] Measured with FPIA-3000S, Sysmex.
[d] $D$[4,3]: volume moment mean diameter.
[e] Diameters at the 10%, 50% and 90% of the population distribution.
[f] Heywood diameter: mean diameter of the circles with equal projected area to particles.
[g] Impossible to be measured due to out of detection limit (ODL).
[h] The total weight of balls was unified to approximate 1.2 g.

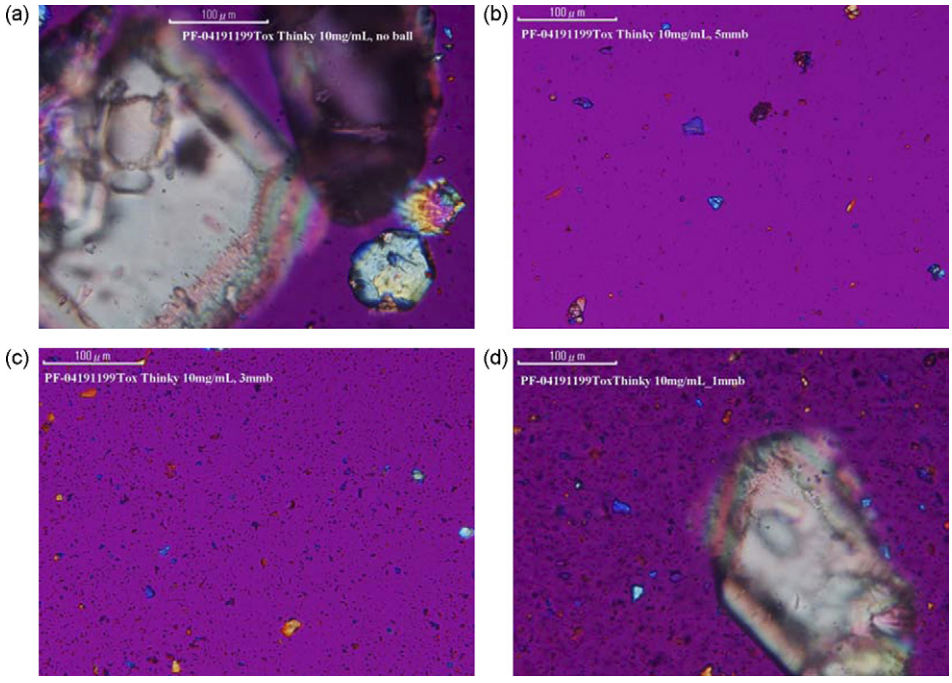


Fig. 1. Polarized light microphotographs of suspension of compound A prepared by the rotation/revolution mixer in combination with various size/quantity of zirconia balls. Bar: 100 μm. (a) no balls, (b) 5 mm Ø × 3 balls, (c) 3 mm Ø × 15 balls and (d) 1 mm Ø × 380 balls.

vehicle (8.623 mL) was added to dilute to the target concentration and the mixer was driven for the final time. The total driving time of the mixer was unified to 4.5 min in these experiments. The microphotographs, PSD and assayed concentration of the resultant suspensions are shown in Figs. 1 and 2, and Table 4, respectively. PSD strongly affects the uniformity of drug content as large particles settle faster than smaller ones according to Stokes' law (Ofner et al., 1996). When zirconia balls were not added to the vessel, the particle size was not reduced at all. This leads to fast sedimentation of the large unmilled particles resulting in poor homogeneity (R.S.D. = 107.9%) with lower content than nominal value in the upper and middle layers of suspension and higher content in the lower layer. On the other hand, driving the mixer with zirconia balls effectively promoted the milling of the particles by crushing the particles with the balls against the inner wall of the vessel. PSD was apparently shifted to smaller size by co-mixing with balls as observed in Fig. 2. The mixing of very high-concentrated paste-like suspension in a small volume of vehicle and some balls is assumed to be the key process to produce the homogeneous suspension with suitable PSD for in vivo oral administration. Especially loading of 15 balls of 3 mm in diameter was most effective among the present trials. The optimum size and quantity of balls (3 mm Ø × 15 balls) also made well-milled homogeneous suspension with higher concentration (60 mg/mL) in Trial #5. This wet milling process with balls produced the homogeneous suspension with the particle size equivalent to the target size provided by the mortar/pestle method ($D_{50} \approx 10$ μm, $D_{90} \approx 30$ μm). The assay data measured by HPLC showed the results were consistent with particle size data. The suspensions (10 and 60 mgA/mL) prepared by the mixer with balls were highly homogenous (R.S.D. < 5%) and contained the nearly intended concentration of API (97.2–98.9%) except Trial #4 (1 mm Ø balls) as shown in Table 4. The milling with the 1 mm diameter balls was not as effective as the unmilled particles were still observed (see Fig. 1). The poor homogeneity (R.S.D. = 11.2%) and lower content (96.5%) than nominal value was also caused by insufficient milling power by with 1 mm diameter balls.

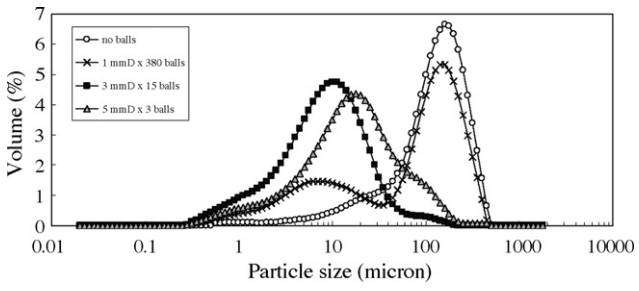


Fig. 2. Particle size distribution profiles of suspension of compound A prepared with the rotation/revolution mixer in combination with various size/quantity of zirconia balls, circle) no balls, cross) 1 mm Ø × 380 balls, square) 3 mm Ø × 15 balls, triangle) 5 mm Ø × 3 balls.

### *3.3. Preparation at a middle and larger manufacturing scale than 1 g loading of the compound*

The manufacturing of a suspension at a scale larger than 1 g powder loading was investigated using compound A (Trials #6–8). In these instances, the formulation at the final concentration was completed in another appropriate container as the final preparation volume is larger than the capacity of the vessel (150 mL). Initially, the powder was milled and dispersed using a small volume of the vehicle in the mixer vessel, and then the thick suspension was transferred by a pipette to a sep-

arate container and finally diluted with remainder of vehicle. This preparation method in which the dispersion and dilution processes were separated enabled the laboratory-sized mixer (AR-250) to manufacture the oral suspension at a larger scale corresponding to the actual preclinical safety study (e.g. suspension over 2000 mL of total volume). In addition, the transferring process is important to visually check that there is no clumping in the suspension and to remove the balls from suspension.

At a loading scale of around 2 g of compound A (Trials #6 and 7), there was a difference observed in PSD of the resultant suspensions, which might be caused by the size and quantity of balls used (see Table 5). The loading with 30 balls of 3 mm in diameter was not effective to reduce the particle size of the suspension. Whereas, mixing with 3 balls of 5 mm in diameter proved to be much more effective in producing equivalent particles to the target size by the conventional method with a mortar/pestle in spite of a half of total ball mass (1.2 g vs. 2.4 g). The 5 mm diameter ball, weighing more than the 3 mm diameter ball, are assumed to be more successful in kneading the large amount of paste-like suspension because the collision impacts with the wall are more forceful during their spinning motion/time in the vessel. At the highest loading of 22.2 g of compound that we tried (Trial #8), the number of balls should increase to disperse the clumping into discrete individual particles. Nine 5 mm diameter balls (around 3.6 g weight) were good enough to make a homogeneous suspension. The appropriate size and quantity of ball could provide a well-dispersed suspension with well milled particles. In addition, it was also found that the manual deaggreration process of un-wet paste by a small spatula was significantly important to enhance dispersion especially in cases of large amounts of API powder loading (see Fig. 3).

The HPLC assay (see Table 5) showed that the concentration and homogeneity of both suspensions (6, 60 mgA/mL) prepared by the mixer in combination with zirconia balls were within in-house criteria (mean 90–100%, R.S.D. $\leq$ 10%) even if the preparation condition was not optimized (Trial #6). This result indicates that there are enough allowable margins to prepare oral suspensions using this method. The suspensions prepared in this experiment have been applied to actual preclinical safety studies without any issues.

Fig. 3. Appearance of manual deaggregation process of un-wet mass of powder by a small spatula.

Table 5
Particle size distribution and concentration/homogeneity of suspensions of compound A prepared with the rotation/revolution mixer

| Trial # | Nominal concentration (mg/mL) | Preparation scale (API, g/suspension, mL) | Balls | Particle size (μm)[a] | | Assayed conc. (mg/mL) | | | Mean (mg/mL) | R.S.D. (%)[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $D[4,3]$[c] | $D_{10}, D_{50}, D_{90}$[d] | Upper | Middle | Lower | | |
| 6 | 6 | 1.8/300 | 3 mm Ø × 30 balls | 88 | 9, 64, 206 | 5.92 | 6.15 | 6.11 | 6.06 | 1.97 |
| 7 | 6 | 2.22/370 | 5 mm Ø × 3 balls | 27 | 2, 14, 60 | NM[e] | NM | NM | NM | NM |
| 8 | 60 | 22.2/370 | 5 mm Ø × 9 balls | 16 | 2, 9, 28 | 61.73 | 59.44 | 62.68 | 61.28 | 2.71 |

[a] Measured with Mastersizer 2000, Malvern.
[b] R.S.D.: relative standard deviation.
[c] $D[4,3]$: volume moment mean diameter.
[d] Diameters at the 10%, 50% and 90% of the population distribution.
[e] NM: not measured.

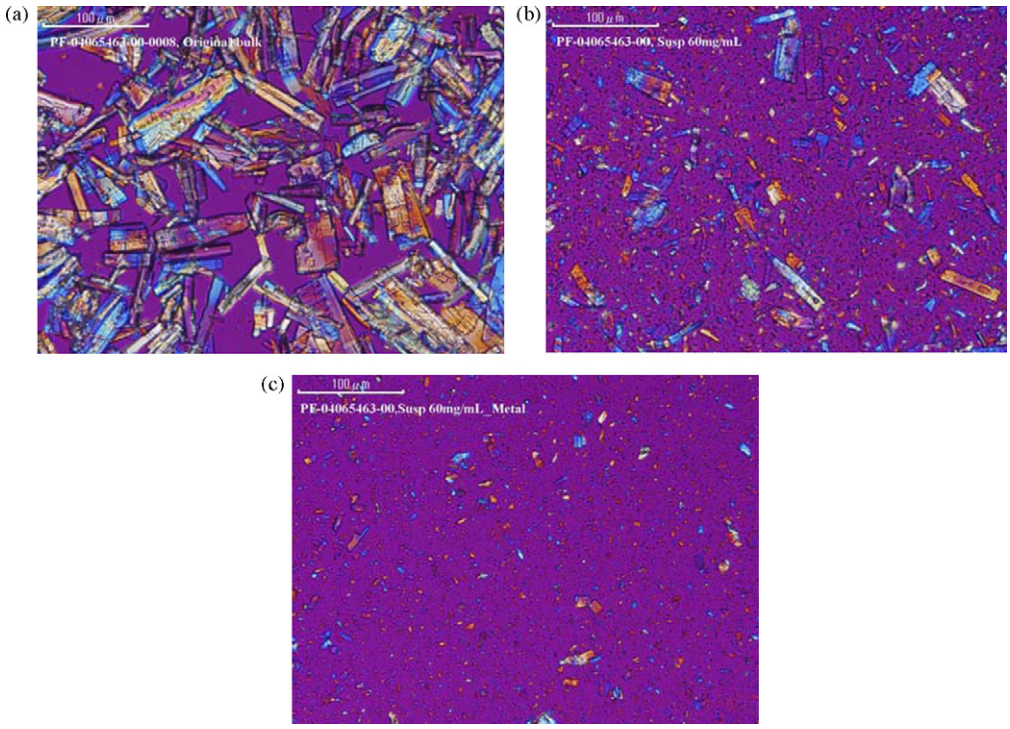


Fig. 4. Polarized light microphotographs of original API powder and suspension of compound B prepared by the rotation/revolution mixer in combination with zirconia balls. Bar: 100 μm. (a) original API powder, (b) suspension prepared in the vessel made of high-density polyethylene (Trial #9) and (c) suspension prepared in the vessel made of stainless steel (Trial #11).

### 3.4. Preparation of suspension with micronized particles

Our previous research indicated that the extent of oral absorption of compound B, which is poorly water soluble, was dependent upon the particle size dispersed in oral suspension. The reduced particle size suspension with $D_{50}$ of not more than 10 μm, called micronization, was required to attain a sufficient oral exposure to the animal. Therefore, the feasibility of the mixer with balls was investigated to produce a finely milled (micronized) suspension. 0.1% Tween 80 was added to the standard oral vehicle of 0.5% MC because compound B is too hydrophobic to be evenly dispersed. Three manufacturing trials with 600 mg/10 mL scale were executed in the following modes: Trial #9: standard driving condition, Trial #10: long driving time, Trial #11: vessel made of stainless steel (Table 2).

The PLM photographs and particle size measurements showed that the rectangular coarse particles of original crystals (20–200 μm in length) were effectively milled with the mixer and balls (see Fig. 4, Table 6). However, the milling effect by the standard procedure established so far (6 min driving time as total, HDPE vessel) was still insufficient to produce a micronized suspension (Trial #9). The preparation at Trials #10 and #11 indicated that both the prolonged driving time of the mixer and the use of a stainless steel (SS) vessel effectively

Table 6
Particle size distribution and concentration/homogeneity of suspensions of compound B prepared with the rotation/revolution mixer. Nominal concentration: 60 mg/mL. Preparation scale: 600 mg/10 mL. Balls: 3 mm Ø × 15 balls

| Trial # | Total driving time (min) | Vessel | Particle size (μm) | | | | Assayed conc. (mg/mL) | | | Mean (mg/mL) | R.S.D. (%)[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Laser diffraction[b] | | Image analysis[c] | | Upper | Middle | Lower | | |
| | | | $D$[4,3][d] | $D_{10}$, $D_{50}$, $D_{90}$[e] | Heywood[f] | $D_{10}$, $D_{50}$, $D_{90}$[e] | | | | | |
| – | Original API powder | | 47 | 8, 32, 100 | 105 | 49, 105, 166 | – | – | – | – | – |
| 9 | 6 | HDPE[g] | 20 | 2, 11, 49 | 37 | 7, 32, 72 | 62.12 | 62.31 | 63.45 | 62.63 | 1.15 |
| 10 | 14.5 | HDPE[g] | 14 | 2, 8, 33 | 18 | 3, 11, 45 | 63.22 | 63.38 | 63.76 | 63.45 | 0.44 |
| 11 | 6 | SS[g] | 11 | 1, 6, 26 | 12 | 2, 9, 28 | 61.26 | 61.29 | 61.13 | 61.23 | 0.14 |

[a] R.S.D.: relative standard deviation.
[b] Measured with Mastersizer 2000, Malvern.
[c] Measured with FPIA-3000S, Sysmex.
[d] $D$[4,3]: volume moment mean diameter.
[e] Diameters at the 10%, 50% and 90% of the population distribution.
[f] Heywood diameter: mean diameter of the circles with equal projected area to particles.
[g] HDPE: high density polyethylene, SS: stainless steel.

Table 7
Summary of preparation conditions of oral suspension using the rotation/revolution mixer (AR-250) in combination with zirconia balls and instructions for formulation preparation

| API, loading (=$X$ g) | Vessel[a] (mL) | Balls, loading | Vehicle addition + Driving[b] | | | |
|---|---|---|---|---|---|---|
| | | | 1st step (mL) | 2nd step (mL) | 3rd step (mL) | Final step |
| $0 < X \leq 0.05$ | 150 | 3 mm Ø × 15 balls (1.2 g) | 0.2 | 0.5 | – | Less than quarter of total vehicle |
| $0.05 < X \leq 0.5$ | 150 | 3 mm Ø × 15 balls (1.2 g) | 0.3 | 1 | – | Same as above |
| $0.5 < X \leq 1$ | 150 | 3 mm Ø × 15 balls (1.2 g) | $X$ | $0.5X$ | – | Same as above |
| $1 < X \leq 3$ | 150 | 5 mm Ø × 3 balls (1.2 g) | $0.8X$ | $0.2X$ | $0.2X$ | Same as above |
| $3 < X \leq 10$ | 150 | 5 mm Ø × 6 balls (2.4 g) | $0.6X$ | $0.2X$ | $0.2X$ | Same as above |
| $10 < X \leq 30$ | 150 | 5 mm Ø × 9 balls (3.6 g) | $0.6X$ | $0.2X$ | $0.2X$ | Same as above |
| $30 < X \leq 60$ | 250 | 5 mm Ø × 12 balls (4.8 g) | $0.6X$ | $0.1X$ | $0.1X$ | Same as above |

[a] HDPE vessel is used as a standard. Stainless steel vessel is alternative to prepare fine-milled suspension.
[b] The process of addition of vehicle and driving the mixer should be repeated three or four times.
Standard operational procedure:

(1) For each dose, weigh preset amount of API powder into a vessel. This step can be done in advance.
(2) Put preset size and quantity of zirconia balls (refer to above Table) into the vessel.
(3) Add preset volume of vehicle calculated from powder weight into the vessel and cover with a lid.
(4) Weigh the adapter plus the vessel containing bulk, vehicle and balls and set counter weight on the mixer.
(5) Set the vessel into a mixer and run the mixer at the fixed driving condition: mixing mode for 1 min → de-foaming (deaeration) mode for 30 s.
(6) Agitate the contents manually using a micro-spatula if un-wet aggregated mass (clumping) still remains; especially in the case of a larger loading more than 10 g of powder.
(7) Repeat steps 3–6 once or twice until solid material is fully wet and evenly dispersed. Homogeneous suspension should be obtained at this stage. Take care that the temperature of vessel keeps almost ambient.
(8) Add the appropriate volume of vehicle (less than quarter of total) while rinsing the spatula. Finally, run the mixer to rinse the adhesive paste from the wall and balls.
(9) Transfer the suspension using a pipette into an appropriate container.
(10) Add the appropriate volume of vehicle while rinsing the compound still adhered to the wall. Run the mixer to enhance the rinsing action as needed.
(11) Repeat steps 9–10 until all API is transferred completely. Fill-up the suspension with the remainder of the vehicle.
(12) Put a stir bar into the container and keep the suspension stirring for at least 30 min before dosing and during its use.

enhanced the wet-milling. An increase in temperature caused by long driving times of the mixer might restrict the application of some compounds, especially compounds which are heat-sensitive. Therefore, the use of SS vessel is recommended as optimum procedure to obtain the micronized suspension less than 10 μm in mean size with respect to poorly water-soluble compounds.

The assay data measured by HPLC showed that all suspensions prepared were highly homogenous (R.S.D. < 2%) and contained the nearly intended concentration of API (102–106%) as shown in Table 6. However, the suspension prepared with long driving times (Trial #10) had a slightly higher concentration than the others. As mentioned above, the temperature of the vessel rose during long driving. This might cause the evaporation of water during opening a lid. From the homogeneity and concentration points of view, the best way to obtain the fine milled suspension is to use the SS vessel.

### *3.5. Instructions for formulation preparation and proposal of driving conditions of the mixer*

Based on the experimental results above, the operational conditions are set depending on the loading amount of API powder (=$X$ g) as shown Table 7, in which the instructions for formulation preparation are also attached. The powder is milled and dispersed in the vessel (capacity: 150 or 250 mL), then the resultant suspension is transferred and diluted with the remainder of the vehicle in a separate suitable container.

Suspensions of compound C with 10, 30, 100 mg/mL were successfully prepared at 73-mL scale according to the proposed procedure and condition. In addition, suspension of compound D with 10 mg/mL was successfully prepared at 2000-mL scale. These suspensions obtained were successfully administered to the animals and gave enough exposure level in plasma concentration in the actual preclinical safety studies.

## 4. Conclusion

In conclusion, the rotation/revolution mixer in combination with zirconia balls has a strong potential for successfully preparing diverse types of oral suspension such as various physicochemical properties of the compound contained (solubility, particle size), wide-range manufacturing scale and concentration. The novel preparation method established in the present experiment is beneficial in the following aspects:

(1) Quick and easy process: The time-consuming process of the conventional method with a mortar/pestle can be avoided because it takes a total of 4.5–6 min to drive the mixer. Skilled formulators are not required because the quality of suspension is operator-independent and reproducible.

(2) One-pot preparation: The preparation is carried out in a closed container, so the content loss (e.g. evaporation of water, spillage of suspension) is minimized during preparation. In addition, the exposure of operators to compound which is not-well characterized from a toxicological perspective and the cross contamination of formulations are also minimized.
(3) Powerful dispersion, milling and deaeration: This method is applicable to API with agglomerates and a larger particle size >150–250 μm. The deaeration (de-foaming) of the suspension is also completed during process. In addition, the poorly water-soluble compounds with solubility-limited absorption are applicable without any pre-milling process, such as jet-milling because the reduced size suspension with mean size <10 μm can be prepared.
(4) Wide range of total preparation volume: This method is applicable to pharmacological and pharmacokinetic discovery studies as well as preclinical safety studies because the preparation scale is varied from 10 to over 2000 mL. The upper limit is based on maximum API loading in 250 mL vessel (e.g. 50–100 g).

Finally, this novel preparation method is quite useful to prepare oral suspensions for pharmacological, pharmacokinetic and toxicological studies in animals at the discovery and preclinical stages in order to mitigate the disadvantages of the conventional preparation method by a mortar/pestle. In addition, this method has enough potential to expand to a new approach for preparing the extemporaneous formulation (oral solution or suspension) in first-in-human study and/or early clinical studies of candidate of the drug due to its easy and reproducible process.

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