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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.