In Situ Determination of Delavirdine Mesylate Particle Size in Solid Oral Dosage Forms

Jackie G. White[1,2]

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Purpose. The in-situ particle size of delavirdine mesylate in dry mix and tablets was determined.

Methods. Optical microscopy and fluorescence microscopy combined with image analysis were used for qualitative and quantitative measurements.

Results. Using optical microscopy, it was demonstrated qualitatively that fragmentation of the large drug particles was occurring during tablet compression. Quantitative comparisons between dry mix and tablet samples showed that in the dry mix, drug particles remain intact, with particle lengths exceeding 200 μm. In the tablets, no particles longer than 100 μm had been observed. Analysis of multiple tablet lots revealed consistent in-situ drug particle size distributions, regardless of the original bulk drug particle size.

Conclusions. Bulk drug particle size of delavirdine mesylate is not predictive of the particle size in the tablet due to fragmentation of particles during compression. Optical and fluorescence microscopy are valuable tools for probing in-situ particle size in complex matrices.

KEY WORDS: optical microscopy; fluorescence microscopy; particle size; image analysis.

INTRODUCTION

Drug particle size can have a significant effect on bioavailability, particularly for poorly soluble drugs. In assessing this effect, interpretations are often made by evaluating the relationship between bulk drug particle size and *in-vivo* performance. It is well established, however, that the particle size can change during compression, either through fragmentation or aggregation (1–3). Various methods have been used to evaluate the change in particle size including dissolution rate studies and measurement of physical properties such as surface area and tensile strength (3–5). Nishioka *et al.* have developed a method for mathematically estimating the drug particle size distribution using laser light diffraction (6). All of these methods involve estimations or indirect indicators of the particle size.

In this report, the change in the particle size of delavirdine mesylate due to compression is measured directly using optical and fluorescence microscopy. Delavirdine mesylate particles have a distinctive morphology, hexagonal and plate-like. Intact delavirdine mesylate particles and large fragments can be easily identified, even when mixed with tablet excipients. Optical microscopy is used to provide a qualitative evaluation of the change in drug particle size which occurs during tablet compression. Delavirdine also fluoresces naturally, so fluorescence microscopy is combined with image analysis to measure the particle size of the drug in powder mixes and tablets. This novel method makes it possible to make particle size measurement of a single constituent within a complex matrix. The combination of the two methods demonstrates conclusively that delavirdine mesylate particles are being fragmented during compression.

[1] Pharmaceutical Development, Pharmacia & Upjohn, Inc., 7000 Portage Road, Kalamazoo, Michigan 49001.

[2] To whom correspondence should be addressed. (e-mail: jackie.g.white@am.pnu.com)

MATERIALS AND METHODS

Optical Microscopy

Bulk drug and dry mix samples were prepared by suspending the powder in butanol and mixing with a vortex mixer. Compressed tablets were gently broken apart using a mortar and pestle. Some of the tablet lots were coated, so the coating material was peeled away from the core tablet using forceps. The resulting powder was suspended in butanol. A drop of the suspended material was placed on a microscope slide and covered with a cover slip. Analysis was done using a Leitz Metallux 3 optical microscope equipped with a Pulnix TM-745 video camera. The microscope was operated using transmitted light and an objective magnification of 100× (resolution = 1.988 μm/pixel) or 50× (3.846 μm/pixel).

Fluorescence Microscopy

Samples of dry mix and tablets were prepared by sprinkling a small amount of dry powder onto a microscope slide. The tablets had been previously broken apart as described above. Fluorescence images were collected using a Nikon Diaphot microscope and a Spex Digital Imaging System/Dual Wavelength Fluorimeter. The excitation wavelength was 340 nm, the emission was filtered through a 400 nm dichroic cube and the objective magnification was 20×. An image of a 2 mm stage micrometer was obtained for calibration purposes. Image files were stored on optical disk in a pseudo-flat file format, then converted to TIFF format using Image Pro Plus.

Image Analysis

Images of the bulk drug particles were obtained using optical microscopy and a Leica Quantimet Q570C image analysis system. The fluorescence images were also analyzed using the Leica system. The black and white images are captured and stored electronically. Particles are detected in the images by setting a threshold gray level. For images where the particles are dark relative to the background, such as bulk drug images, everything which has a higher gray level (i.e., "darker") than the threshold is detected while everything below the level is not detected. (Note: In the fluorescence images, white features were detected.) Ideally the threshold is set such that all particles are detected without detecting any background interferences. Unfortunately it is virtually impossible to detect all of the particles in their entirety without detecting the background as well. The operator must then perform manual editing operations such as filling in incompletely detected particles, separating overlapping particles and deleting interfering features. Individual particles were defined as those which had clearly defined boundaries. Particles which were overlapped by other particles

or which were obstructed from view were not counted. Because some particles were laying sideways or slightly off the screen, particle area (and therefore, equivalent circular diameter) was not a measurable parameter. Particle length was used instead. Particle length, defined as the length of the maximum particle diameter, is calculated by the image analysis software. For bulk drug lots, more than five thousand particles were detected; for the fluorescence studies on the dry mix and tablets, the number of particles detected ranged between 98 and 722.

The image analysis system uses a parameter called the feature limit to determine the minimum number of pixels needed to define a particle. The feature limit combined with the microscopic resolution determines the minimum particle size detected. All studies were done using a feature limit of 2. For the fluorescence images, a 20× objective was used with a resolution of 1 μm per pixel, so the minimum measured particle size was 2 μm. Two different objectives were used for the bulk drug lots; Lot C1 used a 50× objective and Lots C2-C4 used 100× objectives. The minimum particle size measured for these lots was 8 μm and 4 μm, respectively.

Analyses of the size distributions were performed using Excel 5.0. All distributions are number distributions. The distribution statistics were calculated from the cumulative distributions; for example, D90 values represent the equivalent particle diameter at the 90th percentile for a number undersize distribution.

RESULTS AND DISCUSSION

Qualitative Assessment of Particle Size Using Optical Microscopy

Delavirdine mesylate particles are thin plates which range in length from less than a micrometer to over 900 μm. Most of these plates are less than 20 μm thick. In lots with very large particle sizes, the plates often have a hexagonal or rectangular shape (Fig. 1a). This characteristic particle morphology makes it possible to identify intact delavirdine mesylate particles and large fragments of particles even when mixed with excipients in a formulation. Figure 1b shows a photomicrograph of the dry mix for a lot of 100 mg delavirdine mesylate. There are large intact hexagonal delavirdine particles as well as smaller broken particles. The largest delavirdine particles in this figure are approximately 300 μm in length. In Fig. 1c, the photomicrograph of the crushed tablet made from the same lot of dry mix is shown. There are no large particles which can be identified as delavirdine. There are some smaller particles (length < 100 μm) which may be delavirdine but using optical microscopy alone it is impossible to make a positive identification. Multiple sample preparations and different lots of two different strengths were examined but no intact delavirdine particles greater than 100 μm in length were observed in the crushed tablets. The presence of the large drug particles in the dry mix but not in the tablet demonstrates that mixing does not cause the large drug particles to fracture but that these particles are broken apart during compression. This indicates that the particle size of the drug in the tablet may be considerably smaller than that of the bulk drug, although the extent of the particle size reduction cannot be determined from these data.

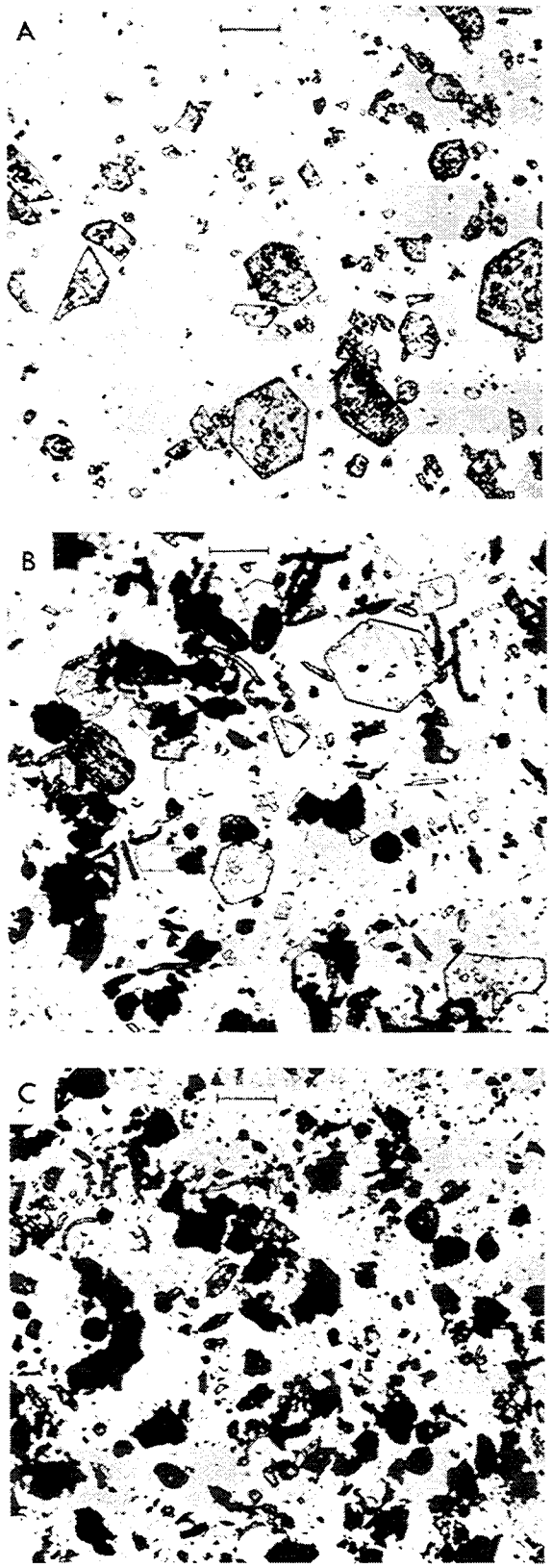

Fig. 1. Optical photomicrographs of (A) Bulk Drug Lot C1; (B) 100 mg Delavirdine Mesylate Lot C1 dry mix; (C) 100 mg Delavirdine Mesylate Lot C1 crushed tablet. 50× objective, Size bar = 200 μm.

Effect of Sample Preparation

In preparing the samples for optical microscopy, the tablets must be broken apart to produce a powder. This is done by gently breaking the tablet using a mortar and pestle. To determine whether the sample preparation method could be causing the delavirdine particles to fracture, a sample of the dry mix was ground in a mortar and pestle. The force used on the dry mix was excessive relative to the force normally applied in breaking tablets. The photomicrograph in Fig. 2 shows the "ground" dry mix. There is some evidence of fracture of the delavirdine particles but there are still drug particles which remained intact and thus can be identified. These photos demonstrate that the fragmentation of the delavirdine particles seen in the tablets cannot be attributed to the manner in which the samples were prepared. The lot shown in Fig. 2 contains bulk drug with a large particle size; other dry mix samples made with smaller bulk drug particle sizes were also evaluated. In all cases, large drug particles were clearly evident in the "ground" sample.

Quantitative Estimation of the Particle Size Using Fluorescence Microscopy

Optical microscopy is a means of obtaining a qualitative look at the delavirdine particles in the dry mix and tablets but it does not provide quantitative information. Under normal circumstances this type of information would be impossible to obtain because of the difficulty of distinguishing between the drug and excipient particles. However, delavirdine is a naturally fluorescent compound and consequently fluorescence microscopy can be used to differentiate between the drug and excipients. Fluorescence microscopy combined with image analysis is used extensively for toxicology studies, so the technology is easily adapted for this application. In Fig. 3, images of dry mix and crushed tablets are shown. The delavirdine particles fluoresce with a white glow while the excipients show up only as a shadowy background. As was seen in the optical microscopy experiments, large (>250 μm) delavirdine particles are present in the dry mix whereas all the drug particles in the tablet sample are less than 100 μm. However the strength of the fluorescence technique lies in the ability to obtain quantitative information. The difference in light intensity in the images allows differentiation between the drug and excipient particles. The image analysis system "identifies" particles in part by detecting difference in light intensity relative to a detection limit set by the operator. After the fluorescent particles are detected, the operator can then edit the detected image to eliminate or separate particles which are overlapping or are not completely in the detection area. After the detected image is edited, the particle length is calculated. Multiple images such as those shown in Fig. 3 are used to determine the particle size distribution of each sample.

Using the image analysis system to analyze the fluorescence images, measurements were made of the particle size distribution in the dry mix and tablets for three lots made with varying bulk drug particle sizes (Table 1). In each case, the

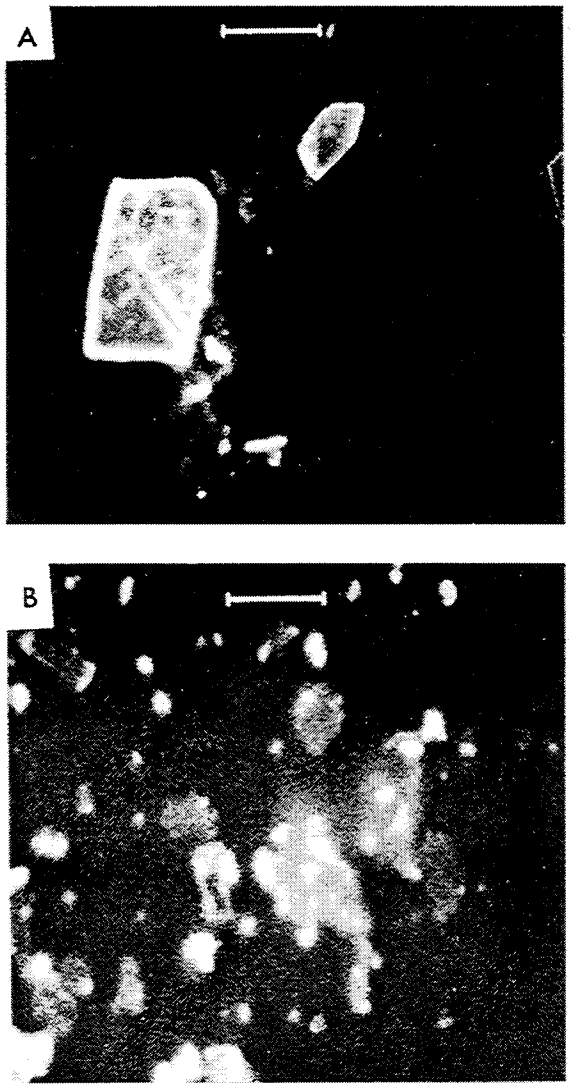

Fig. 3. Fluorescence photomicrographs of 100 mg Delavirdine Mesylate Lot C1. (A) Dry mix. (B) Crushed tablet. 20× objective, Size bar = 100 μm.

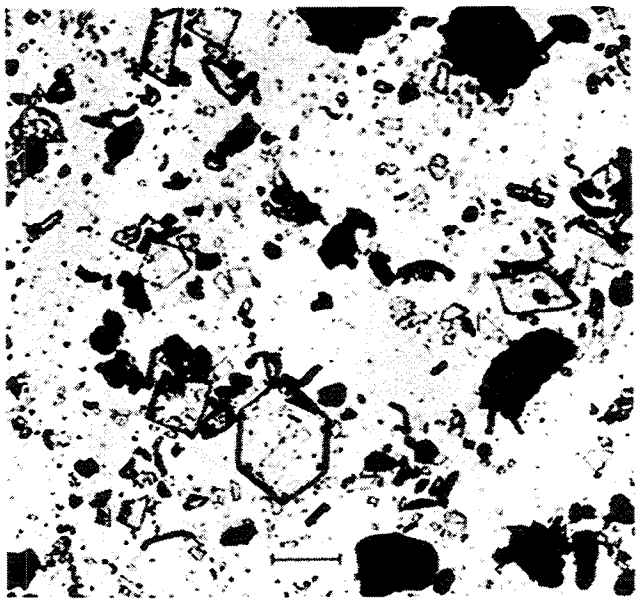

Fig. 2. Optical photomicrograph of 200 mg Delavirdine Mesylate Lot E1, "crushed" dry mix. 50× objective, Size bar = 200 μm.

Table 1. Image Analysis Results for Dry Mix and Tablet Lots

	Dry mix		Tablet	
Lot ID	D90 (μm)	Maximun length (μm)	D90 (μm)	Maximum length (μm)
E2	23	231	27	86
E3	44	212	28	48
C1	71	214	26	89

maximum particle size detected in the tablet is less than half that observed in the dry mix. This is consistent with the observations made using optical microscopy; the large particles have been broken apart during the compression process.

A similar comparison can be made between the bulk drug and the tablet. Four 100 mg tablet lots manufactured using bulk drug of varying particles sizes were compared. Image analysis was used to measure the particle length of drug in the tablets and of the bulk drug (Table 2). In the bulk drug, the maximum particle length ranges from 155 μm to 415 μm; yet in the tablets all of the drug particle lengths are less than 90 μm. The bulk drug used for Lot C1 has a D90 of 58 μm but in the tablet the D90 for the drug is 26 μm. The distribution statistics for the tablets are remarkably consistent, with D90 values varying between 16 μm and 26 μm.

A change in particle size during manufacture could be significant for a poorly soluble drug but delavirdine mesylate has an aqueous solubility (unbuffered water at room temperature) in excess of 300 mg/g. Product performance is not affected by the change in particle size which occurs during compression.

The novel use of optical and fluorescence microscopy for the determination of particle size in a complex matrix represents a significant advance in our ability to understand how manufacturing processes affect the physical characteristics of the drug in the final product. The distinctive properties of delavirdine mesylate provided the opportunity to develop these methods but the possibilities for using this type of technology are many. Obviously the use of fluorescence microscopy is limited to materials which fluoresce but the use of labeling compounds and dyes may provide some options for compounds which are not naturally fluorescent. These studies also emphasize the tremendous power and value of using optical microscopy to examine samples. A wealth of qualitative and sometimes quantitative information can be obtained using this relatively simple technique.

Table 2. Image Analysis Results for Bulk Drug and Tablet Lots

	Bulk drug		Tablet	
Lot ID	D90 (μm)	Maximum length (μm)	D90 (μm)	Maximum length (μm)
C2	16	155	21	71
C3	18	211	16	38
C4	22	175	17	60
C1	58	415	26	89

CONCLUSIONS

Studies conducted using optical and fluorescence microscopy demonstrate that the particle size of delavirdine mesylate is reduced during the direct compression process. The large plates which are observed both in the bulk drug and in the dry mix are fractured when the tablets are compressed. As a result, the particle size of the drug in the tablets is generally smaller than that of the bulk drug and is quite consistent between tablet lots, regardless of the particle size of the bulk drug. Due to the high aqueous solubility of delavirdine mesylate, the change in particle size has no effect on product performance.

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