Drug Development and Industrial Pharmacy, 2011; 37(10): 1208–1216

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.564624

informa
healthcare

RESEARCH ARTICLE

A uHPLC-MS mathematical modeling approach to dry powder inhaler single agglomerate analysis

Justin Pennington, John Lena, Joseph Medendorp, and Gary Ewing

Merck Sharp & Dohme Corp, Summit, NJ, USA

Abstract

Demonstration of content uniformity (CU) is critical toward the successful development of dry powder inhalers (DPIs). Methods for unit dose CU determination for DPI products are well-established within the field of respiratory science. Recent advances in the area include a uHPLC-MS method for high-throughput uniformity analysis, which allows for a greater understanding of blending operations as the industry transitions to a quality-by-design approach to development. Further enhancements to this uHPLC-MS method now enable it to determine CU and sample weight at the single agglomerate level, which is roughly 50× smaller than a unit dose. When coupled with optical microscopy-based agglomerate sizing, the enhanced uHPLC-MS method can also predict the density and porosity of individual agglomerates. Expanding analytical capabilities to the single agglomerate level provides greater insights and confidence in the DPI manufacturing process.

Keywords: Quality-by-design, dry powder inhaler, respiratory, mass spectrometry, content uniformity

Introduction

Demonstration of content uniformity (CU) is critical toward the successful development of drug products, irrespective of the dosage form. For example, tablets, transdermals, suspensions, emulsions, gels, solids, suppositories, and inhalation products each require demonstration of CU[1–3]. Typically, these dosage forms rely on assay values as measured by high-performance liquid chromatography (HPLC). Current regulatory guidance suggests direct HPLC assay-based CU methods of analysis for dry powder inhalers (DPI) as the gold standard method of analysis, performed at sample sizes of one-to-three times the unit dose[2,4,10]. Within the field of DPI product development, there is significant diversity in drug product formulations and delivery systems[5]. For some inhalation products, a single unit dose can be on the order of 1 mg, with individual components comprising <10% of the total drug loading, presenting a unique challenge for analysts to prepare samples accurately and reproducibly. Previously, we have demonstrated a uHPLC-MS-based mathematical approach to CU analysis that greatly increased the throughput of analysis to support the incorporation of quality-by-design (QbD) principles to pharmaceutical development. The method was designed to remove the dependency on accurate sample weight and to increase the overall throughput of the uniformity analysis[6]. The approach was further expanded for use as an at-line process analytical technology (PAT) tool to gain a greater understanding of blending operations[7].

A single unit dose of the Twisthaler® DPI platform consists of stabilized agglomerates of spherical clusters that are ~500 μm in diameter. The agglomerate particles contain a blend of micronized primary particles in fixed ratios[8]. Although CU at the unit dose level has been well-established and its effects studied and understood, a method capable of determining the CU, weight distribution, and other bulk physical properties at the single agglomerate level will provide valuable insights into the manufacturing process. A typical unit dose of 2 mg contains a single dose composite of approximately 50–80 agglomerates. Probing CU at the single agglomerate level allows for determination of whether unit dose uniformity is achieved through the summation of non-uniform

Address for Correspondence: Justin Pennington, Merck, Respiratory Product Development, Summit NJ 07901, USA. Tel: 908-473-6605. E-mail: Justin.pennington@merck.com

(Received 09 December 2010; revised 15 February 2011; accepted 15 February 2011)

agglomerates or whether the manufacturing process produces agglomerates that themselves are uniform.

The uHPLC-MS CU method accurately predicts the analytical sample weight when the volume of dissolution was both known and held constant[6]. The percent agreement between the values was utilized as a metric to demonstrate the suitability of the uHPLC-MS-based approach to uniformity analysis[6]. By accurately controlling the volume of sample dissolution to much lower volumes than used for the unit dose analysis, it is possible to determine the weight of individual agglomerates. Due to the high-throughput nature of the method, it is therefore possible to readily determine weight-based distributions of agglomerates, which through other direct measurement approaches would be tedious and prone to error.

As an additional means of bulk physical characterization, optical microscopic analysis was coupled with the individual agglomerate weight to determine the spherical volume of the agglomerates. This allowed for rapid estimation of individual agglomerate density and porosity, which are closely linked to performance of the final product. Since the agglomerate particles are generally spherical in nature, their volume can be mathematically estimated from their cross-sectional area[9]. Bulk property methods such as loose or tap bulk density provide essential information related to the packing of the agglomerate particles. However, development of an analytical methodology for determination of single agglomerate density provides a unique opportunity for insights into the physical properties of the individual agglomerates.

A single agglomerate uniformity method will provide a means for rapid, reliable, and high-throughput determination of CU, weight-based distributions, and density and porosity estimates for agglomerate-based DPI products. In addition, a method capable of determining the CU at the single agglomerate level will provide insights into whether the Twisthaler® DPI platform consists of fundamentally uniform agglomerate particles.

Materials and methods

Materials

The samples utilized for the current study were chosen to demonstrate the extremes of uniformity analysis. The three components of the drug product used in this study will be referred to as carrier (80%), drug substance A (DS-A: 5%), and drug substance B (DS-B: 15%). Details of the formulation and batch production will not be provided in this manuscript as the analytical methods will be the primary focal points of the current discussion. Acetonitrile (optima grade), methanol (optima grade), formic acid (FA), trifluoroacetic acid (TFA), and glacial acetic acid (HPLC grade) were obtained from Fisher Scientific (Pittsburgh, PA).

Instrumentation and chromatography

A Waters (Waters Corporation, Milford, MA) Acquity™ BEH C18 (1.7 µm particle size, 2.1 cm × 50 mm ID) column was used for all separations. The mobile phase consisted of 0.05% FA in 95% water:5% ACN (solvent A) and 0.05% FA in 95% ACN:5% water (solvent B). A Waters Acquity™ Ultra Performance LC-MS System consisting of the following components was utilized for data collection: Acquity™ Binary Solvent Manager, Acquity™ Sample Manager, Acquity™ UPD Photo Diode Array Detector, and a MicroMass Quattro Micro™ mass spectrometer fitted with a Z-spray electrospray source.

UHPLC-MS analysis

The chromatographic separation comprised of a gradient with an initial condition of 5% solvent B, followed by a linear rise to 95% solvent B over 2.0 min with a 1 min allowance for re-equilibration to starting conditions. A constant 0.7 mL/min flow rate with an isothermal column temperature of 40°C and a 2 µL partial loop injection were used. The MicroMass Quattro Micro mass spectrometer was coupled in-line with the chromatography system with a 50:50 flow split at the MS source. MS detection was accomplished through segmented single-ion recording (SIR) to detect only the ion of interest at specified times in the chromatographic run.

Microscopy and image analysis

Agglomerates were imaged using transmission microscopy (Olympus BX60 5× objective, Diagnostic Instruments Inc., SPOT Insight Mosaic 3.2 camera with SPOT 4.7 acquisition software). From the images, spherical volumes were calculated using two methods. The first approach uses the longest dimension, as measured across the agglomerate, to calculate the volume according to $4/3\pi(d/2)^3$. The second approach uses an automated Matlab image processing routine for edge detection, image binarization, and area determination through pixel counting. Based on the calibrated dimensions of the field-of-view and the number of pixels, the single pixel area of the CCD camera was determined to be 1.48 µm^2. Pixels were selected as either being related to the agglomerate or background through the careful selection of thresholding criteria. The circle equivalent radius was calculated from the formula: Area = πr^2, where area = number of pixels × (area per pixel)^2. The spherical volume was calculated from the circle equivalent radius according to $4/3\pi r^3$. A comparison of values between the spherical volumes from the two methods demonstrated a high level agreement (>90%) and further demonstrated that the agglomerates are highly spherical in nature with aspect ratios of ~1. The circle equivalent radius approach for determination of spherical volume was used for all subsequent analysis.

Results and discussion

Development of a single agglomerate uniformity method

When expanding the uHPLC-MS method for CU to individual agglomerates, there was a significant challenge

related to the handling and preparation of samples due to the microscale nature of the technique. Typical agglomerates weigh <100 µg and have particle sizes <1 mm. To overcome the challenge, a method of sampling was developed that utilized the tip of a modified needle for direct sampling of individual agglomerates from the bulk material with the aid of visual magnification. To remove analytical bias, a large number of samples needed to be evaluated. For the current study, a sample size of 1000 or more was determined to be sufficient to demonstrate the utility of the uHPLC-MS CU approach to single agglomerate analysis, corresponding to roughly twice the mass typically analyzed in unit dose applications.

In order to enable high-throughput analysis, it was essential to limit the number of handling steps in the analytical procedure. To accommodate the large number of samples, the sampled agglomerates were directly loaded into a 96-well plate format for subsequent dissolution in 100 µL of sample solvent. The samples were directly injected onto to the uHPLC-MS system without further dilution. An injection volume of 2 µL was chosen to ensure that the sample concentrations would fall within in the calibrated range of method.

The previously reported uHPLC-MS unit dose CU method was designed to bracket a specified range of analytical sample weights centered on a target 2 mg unit dose. Through the use of volumetric approximation, the weight of the resulting analytical samples did not vary significantly from this target. As a result, the calibration covered a relatively narrow range of 25–200% of the target analytical concentration of 0.2 mg/mL. With this narrow calibration range, it was possible to simplify the mathematical calculations through inverse polynomial fitting. To ensure accuracy and proper polynomial bracketing, the calculated analytical sample weight was utilized as a suitability requirement to ensure that the result was within the calibrated range[6].

When the method was expanded to analyze individual agglomerates, it became necessary to cover a broader calibration range. This broader range accounted for the analytical sample weight covering the entire distribution of agglomerates present in the sample set. In addition, the potential for large variations in percent composition at the single agglomerate level warranted a larger range to ensure proper polynomial bracketing. A standard concentration ranging from 0.01 to 200 mg/mL allowed for only the most extreme outliers to deviate beyond the bracketing standards. To ensure the accuracy of the resulting data, any points that deviated outside of the calibrated range were excluded from further analysis. No samples from a uniform batch were excluded via this criterion, and <5% of the samples from a non-uniform batch were excluded. Evaluation of the chromatograms revealed that all excluded points resulted from particles containing very high levels of a single component causing significantly overloaded chromatographic peaks. Furthermore, optical microscopy confirmed that the outliers were not the result of agglomerate size.

Although adjustments to injection or sample dilution volume could be used to bring the outliers into the calibrated range, it was found to be deleterious as further dilution would cause a large number of agglomerates to be below the limit of quantitation for the carrier component due to its low electrospray ionization efficiency. This would result in the exclusion of a significantly larger number of agglomerates from both the uniform and non-uniform batches. To coincide with the calibration range that covers 5 orders of magnitude, it was necessary to calculate the final mathematical results through standard polynomial fitting with conventional root analysis. For the unit dose method, the narrow calibration range resulted in polynomial fitting by either the inverse or root analysis approaches providing comparable data. All other method characteristics were as previously reported for the unit dose level uHPLC-MS CU method[6].

Single agglomerate uniformity

Through implementation of the modifications described in the previous section, the percent composition of each component in individual agglomerates was determined. Due to the high-throughput nature of the method, the determination of uniformity was accomplished on many replicates in a short period of time. For perspective, it takes ~15 min to prepare a 96-well plate of individual agglomerates for analysis with a 3-min uHPLC-MS run time per sample. To demonstrate the utility of the approach, twelve 96-well plates were prepared and analyzed resulting in 1152 samples per batch. The total number of agglomerates tested is roughly twice the total combined mass that typically tested for the unit dose application. In this way, it was possible to evaluate how the sample scale impacts the relative standard deviations (RSD) of the batch manufacturing process and to what level uniformity is achieved.

To demonstrate the utility of the method in determining differences in uniformity, two test batches were selected including a uniform and a non-uniform batch. Both batches are from the same development program and are identical in composition and starting materials (80% carrier, 5% DS-A, 15% DS-B). The uniformity differences arise from optimization of the manufacturing process during development and are outside of the scope of the current study. The uniform batch was determined to have unit dose uniformity values of 0.6%, 7.4%, and 2.2% RSD for carrier, DS-A, and DS-B, respectively. Values that are below 10% RSD are considered to be uniform at the unit dose level for the purposes of the current study. The non-uniform batch was determined to have RSD values of 1.6%, 25.8%, and 4.4% for carrier, DS-A, and DS-B, respectively, thus resulting in the assessment of non-uniformity. The higher RSD values for DS-A in relation to DS-B and the carrier were consistent with expectation due to its relatively low percent composition (5%) in relation to the other components.

The uniform and non-uniform batches were subjected to single agglomerate uniformity analysis on 1152 individual agglomerates. The method demonstrates significant

differences between the batches with low deviation for DS-A in the uniform batch (Figure 1A, 16.9% RSD) and relatively large deviation for DS-A in the non-uniform batch (Figure 1B, 173.7% RSD). The data are presented in rank order of agglomerate size showing that the percent composition is relatively unaffected by the particle size of the agglomerate. The cumulative mass plot reveals a median particle weight of ~65 µg in either the uniform or non-uniform batch. It is also of interest that deviations from uniformity are not restricted to only the smallest agglomerates in the batch. For the non-uniform batch, the variability is spread across the weight range of the agglomerates. In addition, the data also show that there is no significant difference in the variability of DS-B between the uniform (13.3%) and non-uniform (12.3%) batches. However, as previously described there are significant differences in the variability for both DS-A and the carrier thus indicating potential interaction between the carrier and DS-A as likely the source of the variability between batches.

Optical microscopy and estimation of spherical volume

Microscopy images of single 96-well plate of agglomerate samples from both the uniform and non-uniform batches are shown in pictorial composite form in their well plate locations in Figure 2. This figure demonstrates the particle size range and spherical nature of the particles, both of which are highly similar between the two batches.

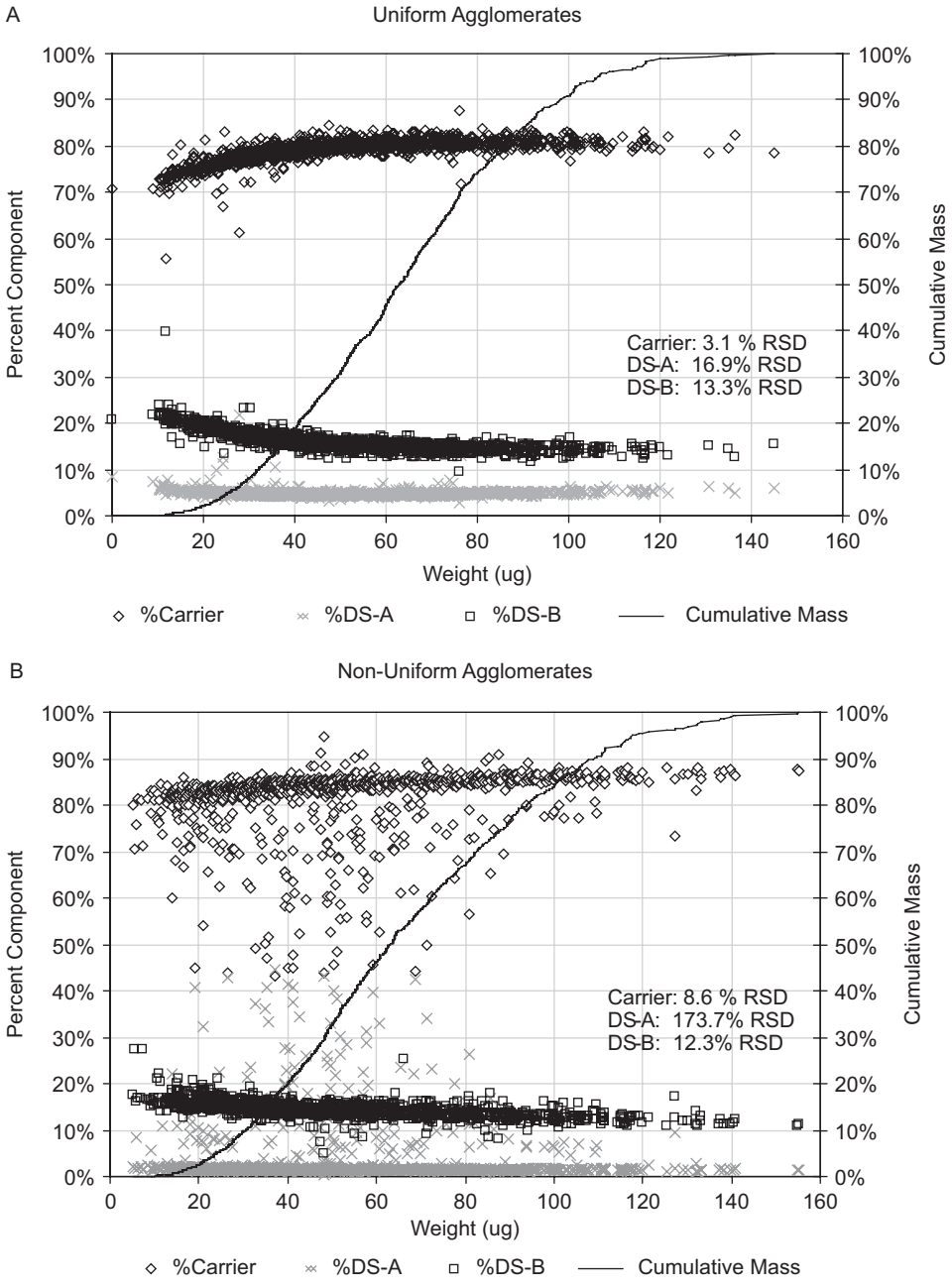

Figure 1. Content uniformity analysis demonstrating the percent component of a typical uniform batch (A) and a non-uniform batch (B) at the single agglomerate level for 1152 individual agglomerates per batch.

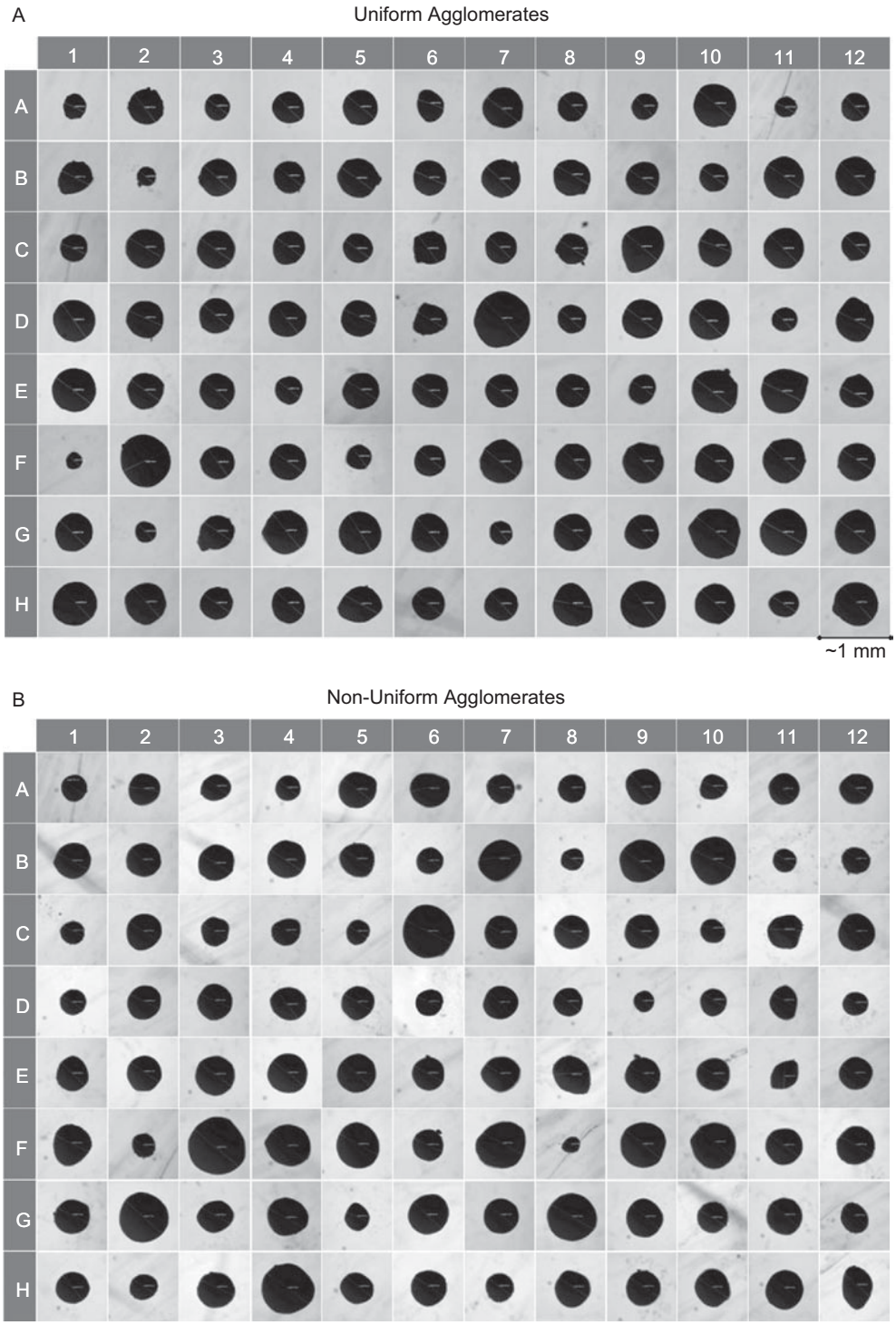

Figure 2. Optical microscopic images of the 96 samples in their uHPLC-MS well plate locations.

When comparing the particle size with the calculated agglomerate weight, it was necessary to convert the two-dimensional agglomerate image to a three-dimensional representation of spherical volume. The approximation of spherical volume using the longest or average diameter can cause ambiguity and can vary based upon the selection of the value. Additionally, it does not take into account any deviation from sphericity.

A Matlab image processing routine was used to calculate the area of the agglomerate through an automated approach and convert to spherical volume directly from the area value. By converting the images to their binarized mathematical representations in Matlab and directly reading pixel values, the inherently subjective nature of image analysis was removed. Some care was taken in selecting the appropriate threshold values to ensure consistent edge detection from image to image within the data sets. Since the camera was focused on spherical agglomerates, some blurrings occur toward the agglomerate edges, giving a slight hazy appearance. The

threshold was selected such that the outer edge of the agglomerate haze was the boundary condition. A binarized visual representation was generated and compared with its original transmission microscopy image to ensure the program appropriately measured the agglomerate.

Relationship of agglomerate weight and particle size

To characterize the single agglomerate method's ability to determine accurately the weight of individual agglomerates, there was a need for a comparative primary analytical method. One approach employed was to weigh a 2-mg sample and count the number of agglomerates present. A typical unit dose was found to contain between 50 and 80 agglomerates resulting in an average weight of individual agglomerates between 25 and 40 μg. An additional approach was to evaluate the relationship between calculated agglomerate weight and spherical volume.

As previously discussed, an additional output of the uniformity method is the prediction of the analytical sample weight. For the unit dose scale, this weight was used for the purposes of system suitability to ensure that the sample was within the calibrated range of the instrument. In the case of a single agglomerate analysis, this calculated weight represents the weight of the individual agglomerate. The method allowed for a very rapid determination of agglomerate weight, generating enough data to derive an agglomerate weight distribution in a fraction of the time that would be required for microbalance-based approaches. The

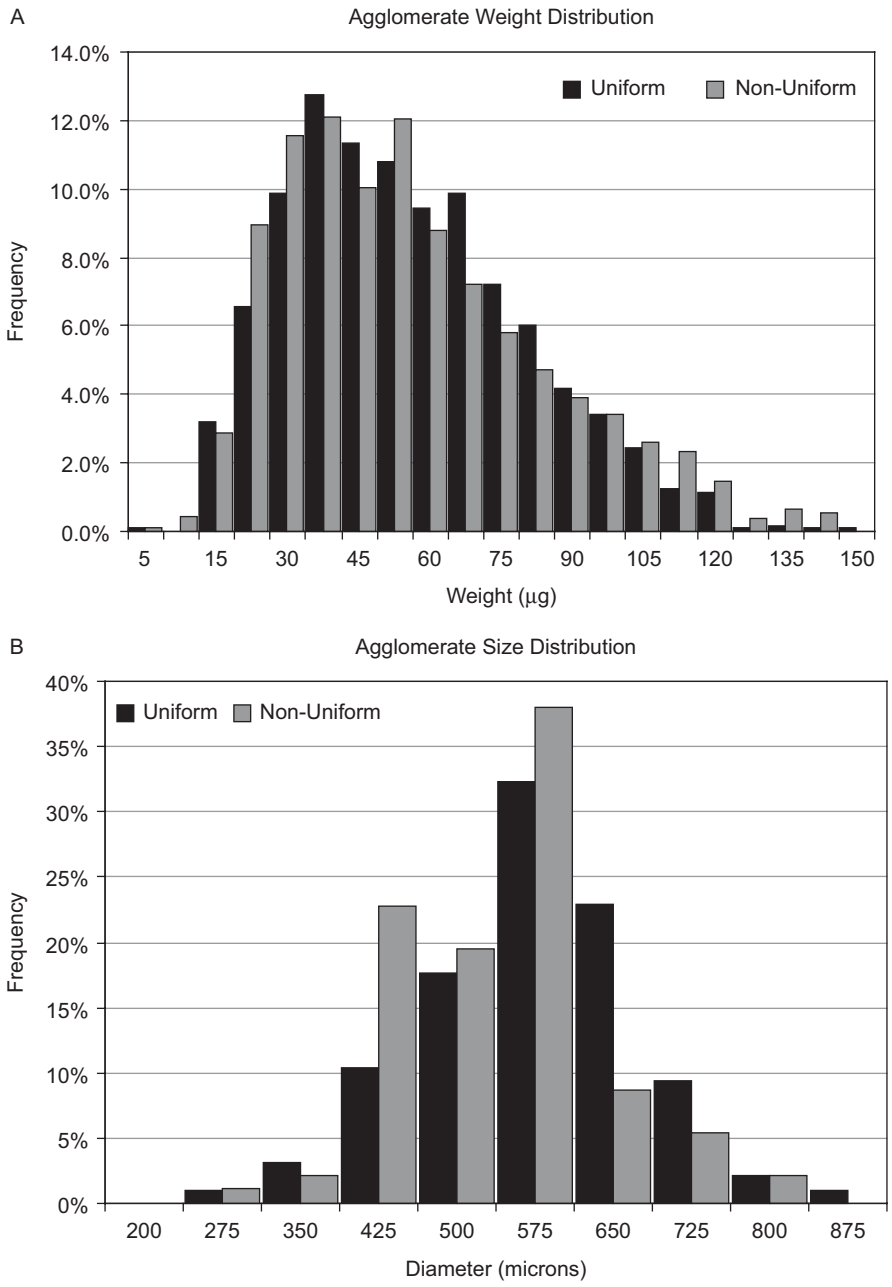

Figure 3. Agglomerate weight (A) and size (B) distributions for both a uniform and non-uniform batch demonstrating the robustness of the analytical method and batch manufacture.

distribution of agglomerate weights demonstrates a small deviation in normality, as shown in Figure 3A. However, it is of interest that the uniform and non-uniform batches show very similar weight distributions thus demonstrating the robustness of the agglomeration process in producing consistently sized agglomerates.

The distribution of agglomerate spherical volume, as determined in the previous section, is shown in Figure 3B. In similar fashion to weight distribution, there is very little difference in the overall spherical volumes observed between the uniform and non-uniform batches. This data further evidenced that in regard to manufacturing of reproducibly sized agglomerates the agglomeration process is both robust and not specific to the final uniformity of the blending operations. However, as previously discussed there is need for optimization of the blending operations to ensure that the resulting agglomerates are uniform in percent composition, which is vital for the final performance of the product.

The relationship between particle weight and spherical volume was further evaluated. As shown in Figure 4, except for a few outlier agglomerates, there is an increase in the weights of the agglomerates with increased spherical volume for both the uniform and non-uniform batches. This relationship would not be present if the calculated agglomerate weight from the method were not trending in the proper fashion. There is a small amount of curvature present indicating a potential decrease in particle density with increased spherical volume. Although there are many potential causes for this behavior, further studies will be required to determine if the effect is real or caused by mathematical deviations in the model.

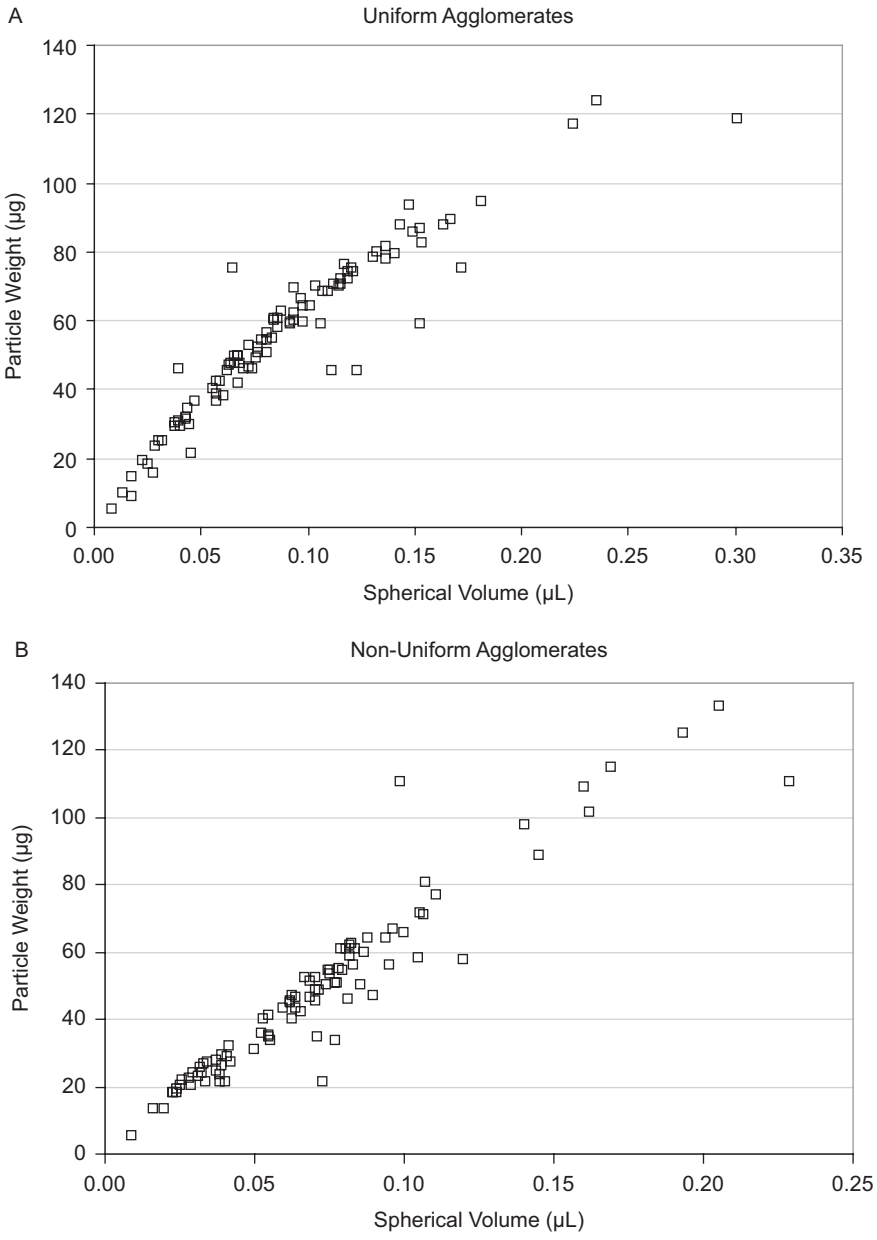

Figure 4. Correlation between the spherical volume (calculated from optical microscopy) and particle weight (determined from the uniformity method) for both a uniform and non-uniform batch.

Estimation of agglomerate density

Many different approaches are used to determine the density of powder-based products including bulk and tap density. Each of these approaches includes void spaces that are present in the bulk powder. By taking the unit-converted values of particle weight (g) and dividing by the spherical volume (mL), it is possible to approximate the true density of the individual agglomerates. For reference purposes, the tap densities of the uniform and non-uniform batches are 0.34 and 0.35 g/mL, respectively. The individual density values for each of the agglomerates are shown both as a function of particle size in Figure 5A, and in histogram form in Figure 5B. A decrease in particle density can be observed with increases in particle size with the average density of 0.7–0.8 g/mL for both batches. Considering the density of the largest component, which comprises 80% of the blend, is ~1.5 g/mL, one can estimate the porosity of the agglomerates to be ~50% by comparison with the average density of the particles. In addition, the estimated density value is situated between the tap and true density values as would be predicted.

Conclusion

The method for uHPLC-MS-based CU has been expanded to the single agglomerate level to provide insights into how changes in the manufacturing process influence the individual agglomerates that comprise a typical dose from the Twisthaler® DPI platform. The

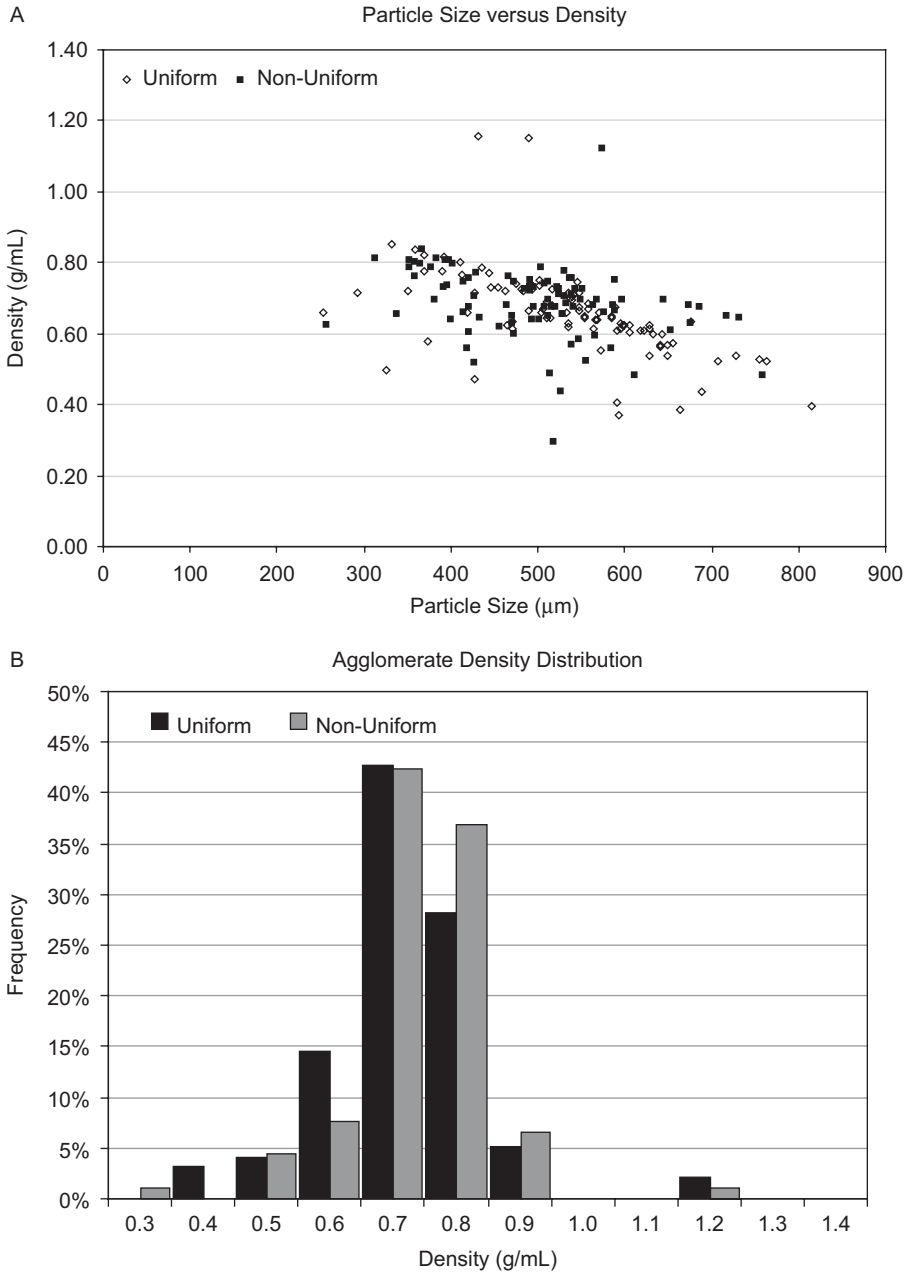

Figure 5. Approximating single agglomerate density for the uniform and non-uniform batches: (A) density as it relates to particle size and (B) density histogram.

method is capable of rapid determination of percent composition and weight distributions on thousands of representative samples at an analytical scale that was previously unachievable by traditional assay techniques. Furthermore, when agglomerate weight calculations are coupled with optical microscopy it is possible to rapidly estimate the density and porosity values of individual agglomerates, properties that are closely linked to performance of the final product.

The method provides a tool for the analysis of dry powder agglomerates and provides valuable information for formulation and process development. The method demonstrated that unit dose CU for Twisthaler® agglomerates was not the result of a mixture of non-uniform agglomerates combining to achieve uniformity, but resulted from an optimized manufacturing process that produces fundamentally uniform agglomerates.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

References

1. USP Official. (2008). General Chapters: 905 Uniformity of Dosage Units.
2. U.S. Department of Health and Human Services, Food, and Drug Administration, Center for Drug Evaluation, Research. (2003). Guidance for Industry: Powder Blends and Finished Dosage Units-Stratified In-Process Dosage Unit Sampling and Assessment.
3. Senderak ET. (2009). Content uniformity acceptance limit for a validation batch–suppositories, transdermal systems, and inhalations. Drug Dev Ind Pharm, 35:735–737.
4. US vs. Barr Laboratories. (1993). 812, F. Supp 458, D.N.J.
5. Son YJ, McConville JT. (2008). Advancements in dry powder delivery to the lung. Drug Dev Ind Pharm, 34:948–959.
6. Medendorp J, Donovan B, Pennington J. (2010). Development of a high-throughput UHPLC-MS-based content uniformity method as a tool for assessing dry powder inhalers. Drug Dev Ind Pharm, 36:762–772.
7. Pennington J. (2010). Determination of content uniformity for dry powder inhaler formulations: UHPLC-MS based mathematical modeling approach as a PAT tool. Respir Drug Deliv 3:667–670.
8. Karpel JP. (2000). An easy-to-use dry-powder inhaler. Adv Ther, 17:282–286.
9. Brewer E, Ramsland A. (1995). Particle size determination by automated microscopical imaging analysis with comparison to laser diffraction. J Pharm Sci, 84:499–501.
10. Berman J. (2001). The compliance and science of Blend Uniformity Analysis. PDA J Pharm Sci Technol, 55:209–222.