Multiscale Tomography: Probing the Nano-, Micro-, and Meso-scale Resolution of Inhalation Powder Structure

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KEYWORDS: dry powder inhaler (DPI), lactose, x-ray computed tomography (XCT), critical material attributes (CMAs), microstructure, Q3 structural equivalence

SUMMARY

Understanding the agglomeration behavior of inhaled formulations is essential to ensure consistent aerosolization performance that maximizes drug deposition in the lower respiratory tract. Although many techniques are currently used to characterize critical material attributes (CMAs) such as the particle size distribution (PSD), these techniques suffer from low resolution, image in 2D projections, or are based on shape assumptions. Bearing in mind the importance of the powder microstructure, this work introduces the use of x-ray computed tomography (XCT) as a non-destructive, multiscale technique for characterizing inhalation formulations. Different grades of inhalation and tableting grade lactose have been analyzed using XCT, with distributions for size (volume weighted) and sphericity extracted and compared with laser diffraction and optical

microscopy. The three-dimensional information provided from XCT provides a more accurate assessment of powder size and shape, demonstrating the promise for XCT as a valuable powder characterization technique that provides information about the powder microstructure, a descriptor now required from the Food and Drug Administration (Q3 equivalence).

INTRODUCTION

Powder formulations for use in dry powder inhalers (DPIs) are commonly composed of a blend of coarse carrier particles (typically α-lactose monohydrate) and micronized active pharmaceutical ingredient (API), with extrinsic lactose fines occasionally added to improve the aerosolization performance [1–3]. The dispersion of the API and the lactose fines on the surface of the coarse carriers influences the de-agglomeration of the particles taking place during aerosolization, thus affecting the fine particle fraction (FPF). Ideally, the aerodynamic size of the deposited particles should be in the range of 1-5 μm [4]; thus it is important to measure accurately the PSD in a DPI formulation. Several techniques are commonly used to evaluate the PSD, including laser diffraction (LD), scanning electron microscopy (SEM), optical microscopy (OM), cascade impaction (CI), Raman microscopy (RaM), and atomic force microscopy (AFM). While these techniques assess CMAs spanning different size scales, dispersion of the particles is needed which involves break up of their formulated bulk structure resulting in loss of information about the powder microstructure. In addition, these techniques do not allow the visualization of the formulated particles in all three dimensions with good resolution, or suffer from approximations due to the non-spherical nature of the particles [5]. To date, a complete description of the microstructure of agglomerated particles for inhalation is lacking.

The microstructure of inhalation powder formulations has become increasingly important, since the Food and Drug Administration (FDA) introduced the concept of microstructural equivalence (Q3) to compliment the qualitative (Q1) and quantitative (Q2) equivalence that a generic product-candidate has to match with the reference listed drug product [6-8]. This paper introduces the use of x-ray computed tomography (XCT) as a novel non-destructive tool to assess the micromeritic properties of powders formulated for inhaled delivery. Data analytical strategies are also required to translate the microstructural information such that XCT data can be correlated to information obtained from existing conventional techniques.

Existing Powder Characterization Techniques

Characterization of powder properties requires knowledge of the particles' behavior across multiple scale levels. At the meso-scale, techniques including pycnometry, porosimetry, or powder rheometry are able to provide a behavioral description of the powder [12], clearly governed by the microstructure of the particles. Brunauer-Emmett-Teller (BET) specific surface area determined by gas adsorption [3, 13, 14] is another relevant technique. At the meso- and micro- scale, LD, SEM, OM, and CI are the most commonly used industrial techniques for determining particle size and shape distributions, while RaM is commonly used to detect chemical composition. In LD, the diffraction of a laser-beam though the dispersed powder sample is used to estimate the size distribution assuming the powder crystals are spherical. However, lactose crystals (known as grains in material science) have a non-spherical tomahawk shape and despite the introduction of an adjustment factor to account for the non-sphericity (which requires prior shape description), it is known that LD measurements are biased [5].

Microscopy approaches, including SEM and OM, provide valuable insight for shape analysis, where powder is dispersed on a slide imaged using either an electron or optical beam. Both methods produce a 2D image which can be processed to determine shape metrics including the aspect ratio, circularity, or circular equivalent diameter. Rapid and automated optical sample analysis of a dispersed powder has also been developed (e.g., Morphologi (Malvern Panalytical, UK)). However, these metrics are heavily dependent on the specific particle orientation when the particle is imaged and may provide very different results from crystal to crystal depending on the particular distribution of crystal orientations on the slide. As such, the latter microscopy techniques fail to provide truly 3D shape information.

In impaction analysis (e.g., using the next generation impactor (NGI)), the powder is dispersed under an airflow and the particles are deposited on different stages according to their aerodynamic behavior, allowing the quantification of the aerosolized fine particles which provides an indication of the extent of de-agglomeration within the formulation. MDRS, a Raman-based technology that combines shape, size, and chemical identification has been used in conjunction with *in vitro* dissolution testing to measure the equivalence of size distribution and chemical composition of aerosolized agglomerates. Dissolution testing itself provides an indirect estimation of the microstructure of formulated powders. The combination of impaction analysis, MDRS, and dissolution testing provides a powerful approach to characterize equivalence of agglomerate and emitted aerosol microstructure between products [8]. However, all of the aforementioned techniques rely heavily on powder dispersion and are destructive to the powder formulation.

Finally on the nano-scale, AFM has proven to be an invaluable tool able to provide topographical and roughness measurements of drug crystals; these have been used to quantitatively assess the cohesive-adhesive balance (CAB) of DPI formulations [15-18]. However, CAB measurements can be very challenging and are limited to inter-particulate interactions at a single particle level.

While the deficiencies of existing techniques are widely acknowledged, the lack of a viable alternative has meant that these methods have become standard size and morphometric tools. INFORM 2020 is a research consortium funded by the United Kingdom's Engineering and Physical Sciences Research Council to integrate advanced imaging, materials characterization, and particle computational modeling approaches to overcome the limitations of existing isolated analytical approaches. This article will describe the development of imaging techniques for inhalation formulations to produce size and shape distributions based on actual 3D characteristics of a powder.

X-ray Computed Tomography

The ability to use x-rays to look inside objects was first discovered by Wilhelm Conrad Roentgen in 1895, with the first medical radiograph being of his wife's hand [19]. Subsequently, x-ray radiographs were increasingly used to provide planar-projected images both for material science and medical applications. In 1973, Hounsfield discovered that depth perception could be recovered from x-ray imaging techniques by combining radiographs taken from different directions [20]. Known as XCT, this process has emerged as an invaluable, non-destructive method for probing internal structures. For example, chest CT scans are routinely used in the diagnosis and assessment of lung disease [21].

XCT has also been used in material science to non-destructively examine the interior of objects [22], to detect cracks in metal alloys [23], or monitor the degradation of thermal barrier coatings [24]. Because the majority of material science samples do not hold radiation exposure restrictions (compared to medical applications), material science XCT can have much longer scan

times and reach up to sub-micron resolution. The richness of 3D information provided by XCT has also been exploited to examine a variety of granular materials, ranging from large rocks in the mining industry [25] to fine powders used in additive manufacturing [26]. For example, XCT has been used to quantify the size and shape of metal powders [27] and rock samples [27], as well as characterization of the internal porosity of sintered beads [29] without damaging or destroying the powder. Despite its potential, applications to pharmaceutical powders have been more limited; particle packing in a model pharmaceutical powder has been investigated [28], and the porosity and internal microstructure of individual granules examined [29, 30]. The small size of inhalation powders presents a challenge for XCT, but improvements in instrumentation provide an opportunity to gain unique insights.

The variety of available instruments means that different scales of XCT are possible in the laboratory. The majority of instruments are known as micro-CT systems, allowing pixel sizes down to 0.5 μm. The exact resolution, i.e., ability to resolve two separate objects, is dependent on a number of factors including the particular material(s) under consideration. However, pharmaceutical powders with crystal sizes in the range 5-1000 μm can typically be quantified. Figure 1A shows the Xradia 520-DCT Versa system (Carl Zeiss Microscopy, USA) which was used in these studies to characterize the 3-dimensional structure of inhalation powder samples. Flexibility in setup geometries mean that a larger field of view can be examined with a coarser resolution or a smaller field of view with a finer resolution, with the field of view equal to the image-size multiplied by the image pixel size. For example, a DPI several centimeters in width can be imaged with a pixel size of 50 μm, or an entire 5 mm capsule containing powder blend can be imaged with a pixel size of 20 μm, or a bulk powder imaged with sub-micron pixel size. Recently, laboratory systems have been designed for nano-scale CT where a resolution of 50 nm can be achieved. For example, the Xradia 810-Ultra system (Carl Zeiss Microscopy, USA) allows an image resolution of 50 nm and 100 nm for maximum sample sizes of 16 μm and 64 μm, respectively [31]. Here, we show how both micro-CT and nano-CT can provide microstructural insight on powder blends.

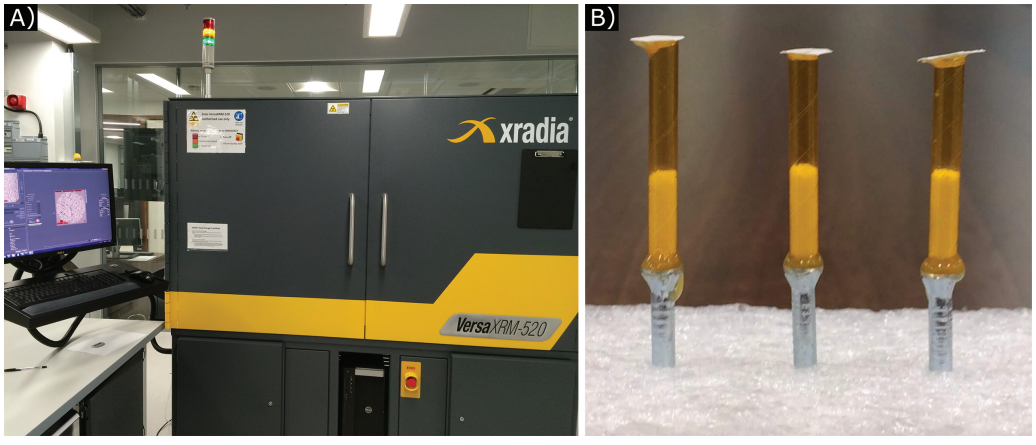

Figure 1. **A) The Xradia 520-DCT Versa XCT system (Carl Zeiss Microscopy Limited, USA). B) a typical sample holder for bulk powders.**

ANALYZING POWDER SAMPLES THROUGH MICRO-CT

Micro-CT can be used to scan bulk powder samples with very simple sample preparation. The sample holder shown in Figure 1B is constructed by gluing lengths of polyimide tubing onto a nail and filling with 1-10 g of powder. Finally, the tube is sealed by gluing a small piece of paper on the top of the tube. Unlike laser diffraction or microscopy, no dispersion of the powder is required, while the non-destructive nature of XCT means that the powder can be re-used for other tests.

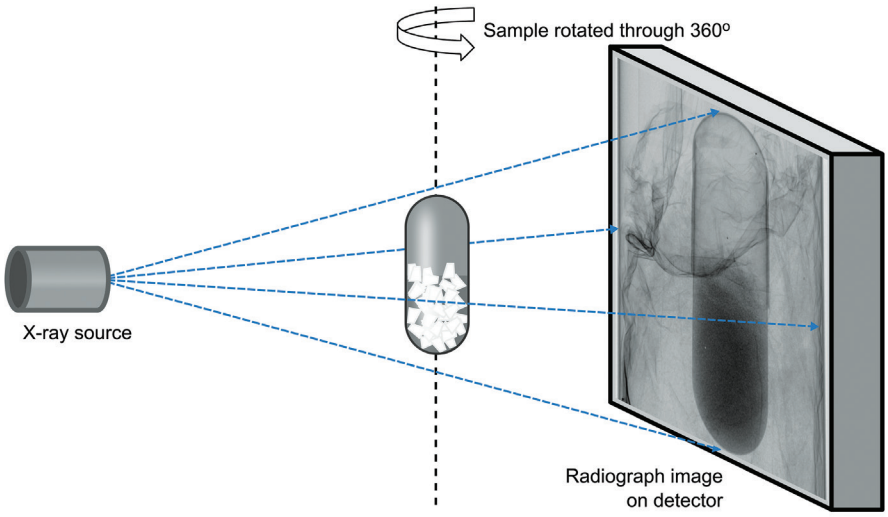

Figure 2. An illustrative set-up for x-ray computed tomography (XCT) of a powder sample contained within a capsule.

An illustrative set-up for XCT to quantify the powder distribution within a capsule, which is a goal of our research, is shown in Figure 2. In laboratory micro-CT machines, an electron tube creates a point source of polychromatic "white" x-rays emanating as a cone. The x-rays are absorbed by the sample according to Beer-Lambert law, with absorption increasing with atomic density. The transmitted x-rays are incident on the detector, where the intensity of the waves is converted to a greyscale shadow image as shown on the detector in Figure 2. The difference between the transmittance of air and the sample provides the contrast. As the transmittance through single crystals can be as high as 95%, and through a bulk powder can still be 75%, this is a particular challenge when scanning lactose powders. The lack of absorption contrast can be compensated by phase contrast, where dark and bright fringes are found at the interface between the powder crystals and air. The combination of the coherent sources, magnification optics and small working distances in the Zeiss Xradia Versa family of XCT systems (Figure 1A), produce such phase fringes in-line making them particularly suitable instruments for scanning inhalation powder samples.

The detector in Figure 2 shows a single radiograph of a capsule, but radiographs from different directions are needed for computed tomography. Whereas in medical XCT, the patient is kept stationary and the source and detector moved, the majority of material science XCT machines

use a stationary source and detector and rotate the sample through 360° about a vertical axis for the different views. The stationary source and detector provide greater physical stability which makes image alignment easier, hence allowing high resolution images. While each individual radiograph has no depth information, the series of radiographs can then be mathematically reconstructed using a filtered back-projection algorithm (Feldkamp-Davis-Kress, [32]). The algorithm correlates the difference in the radiographs with rotation of the projections to form a virtual volume representation of the sample.

Two model powders were used as an illustration in this work, whose particle size distributions given by laser diffraction and bulk densities reported by the manufacturer are given in Table 1. CapsuLac 60, a tableting grade (Meggle, Germany) and Lactohale 100 (DFE Pharma, Germany). Approximately 150 mm^3 of powder was loaded into each sample holder and scanned in the Xradia 520-DCT Versa instrument. The voltage was 80 kV, the power was 7 W and the exposure time was 3.5 seconds for each of the 3201 projections, using 1x detector binning. The source to object distance was 12 mm while the object to detector distance was 14 mm. Due to the differences in crystal size summarized in Table 1, CapsuLac 60 was scanned with a 4× optical magnification and a pixel size of 1.56 μm, while Lactohale 100 was scanned using a 10× optical magnification with a pixel size of 0.64 μm.

Table 1.

Particle size distribution statistics as measured by Laser diffraction analysis for CapsuLac 60 and Lactohale 100 (LH100), with reported powder bulk density values for both materials.

	D_{10}	D_{50}	D_{90}	Bulk density
Sample Name	**(μm)**			**(g/cm^3)**
CapsuLac 60	120	215	338	570 [1]
Lactohale 100	55	134	222	690 [2]

[1] data from supplier. [2] data from [33].

Image analysis, where the final virtual volume is segmented to label important parts of the sample, is an important part of XCT. This is particularly true when scanning inhalation powders, where post-processing is needed to identify each of the individual particles, or each of the parts of an agglomerate. There are a wide range of segmentation techniques, such as morphological operations [34], watershed filling [35] or manual identification, and these techniques are typically used in combination to produce the optimal result for the type and amount of powder scanned. Segmentation is the biggest challenge for accurate quantification, because if two adjacent particles are incorrectly labeled as a single particle, the size and volume would be approximately double the true value; this would additionally impact identification of inter-particulate volumes, and over-estimate the powder density. One virtual cross-section through the reconstructed volume of CapsuLac 60 is shown in Figure 3A, with the segmented image shown in Figure 3B using a different color for each particle. Visual inspection is an important verification check of the segmentation accuracy, and the commercial software package Avizo (Thermo Fisher Scientific, France) is a popular choice since it provides three-dimensional visualization alongside image processing.

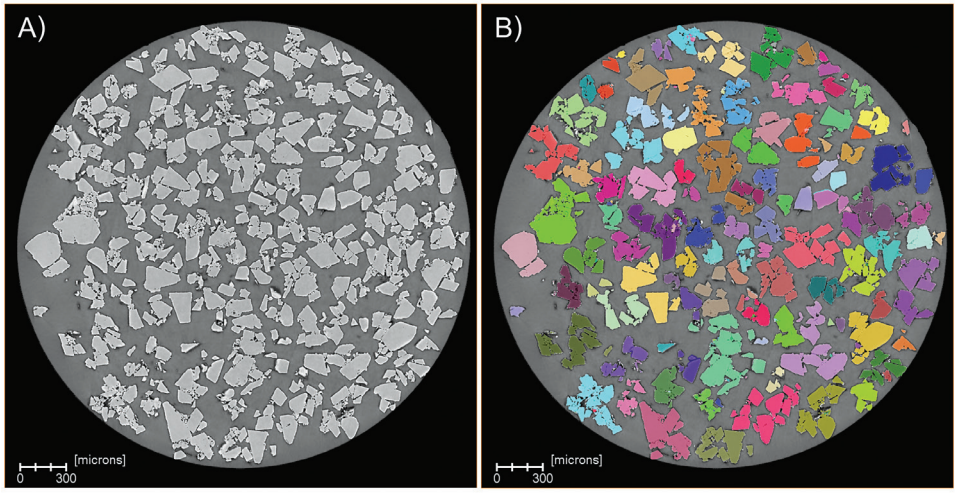

Figure 3. **A) A virtual cross-section through the reconstructed volume of CapsuLac 60. B) the same cross-section with a different color for each identified particle.**

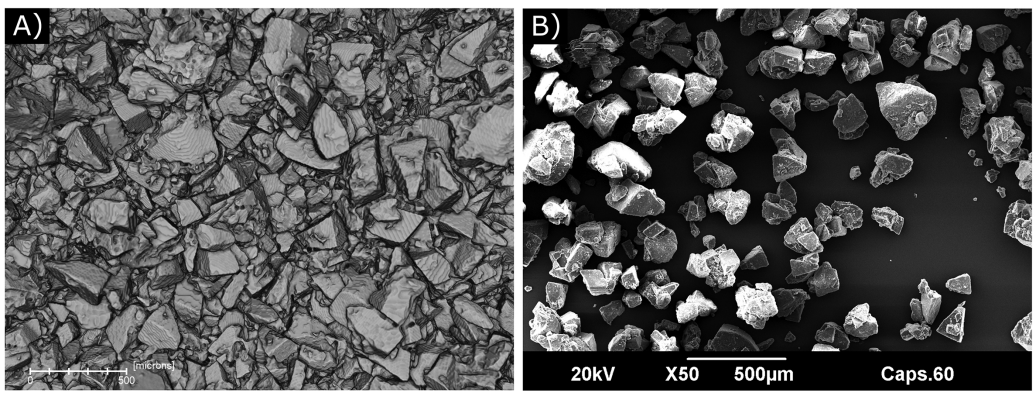

Figure 4. **Particle morphology of CapsuLac 60 characterized using A) x-ray computed tomography and B) scanning electron microscopy. The image in A) is a 3D visualization of the virtual reconstructed volume.**

The final segmented volume allows the morphology to be clearly assessed. Figure 4A shows a three-dimensional visualization of a bulk powder sample of CapsuLac 60 in which the predicted tomahawk shape of lactose is clearly identifiable. This is comparable to the SEM image of a similar magnification in Figure 4B that is currently the standard technique used to describe a powder sample; however, XCT provides a much higher level of definition and detail. In addition, the virtual volume can be rotated and viewed from different orientations.

MESO-SCALE CHARACTERIZATION OF BULK POWDERS COMPARED TO EXISTING TECHNIQUES

It is convenient to summarize the properties of an entire powder sample using mesoscale statistics and statistical distributions. For example, the size distribution curve summarizes the sizes of all of the particles within the sample, while the D10, D50 and D90 percentiles give an indication of the number of fines, the median crystal size and the presence of large agglomerates. Post-segmentation, each of the individual particles within the virtual XCT volume can be scrutinized to calculate the particle volume and surface area, along with shape descriptors such as Feret's diameter [36] and particle sphericity. Distributions produced from this can be directly compared with existing techniques such as laser diffraction and microscopy. This comparison is important as it showcases the advantages of using XCT for bulk characteristics.

Laser diffraction analysis of Lactohale 100 was performed following a previously described protocol [37] using a Sympatec HELOS/RODOS LD unit (Sympatec, Germany) coupled with an ASPIROS dispersing system. The dispersing aperture diameter was 4 mm, the feed velocity was 25 mm/s and the R5 lens was fitted for the measurements with a measuring range of 4.5-875 µm. The ASPIROS glass vials were filled with Lactohale 100 powder, which was then dispersed via vacuum suction. Particle shape analysis of CapsuLac 60 was also determined through OM using a Morphologi G3 (Malvern Instruments, United Kingdom). Approximately 26 mm^3 sample of CapsuLac 60 powder was dispersed onto a glass plate at a pressure of 0.5 bar using the automated sample dispersion unit, with an optical ×5 magnification lens used to image each of the dispersed particles. The resolution range of the lens was 6.5-420 µm, ensuring that both larger particles and agglomerates were captured. The image analysis captures a two-dimensional (2D) image of a 3D particle projected on the glass plate and calculates various size and shape parameters from it; the distribution for the entire bulk is produced from the results of the entire dispersed powder population distributions.

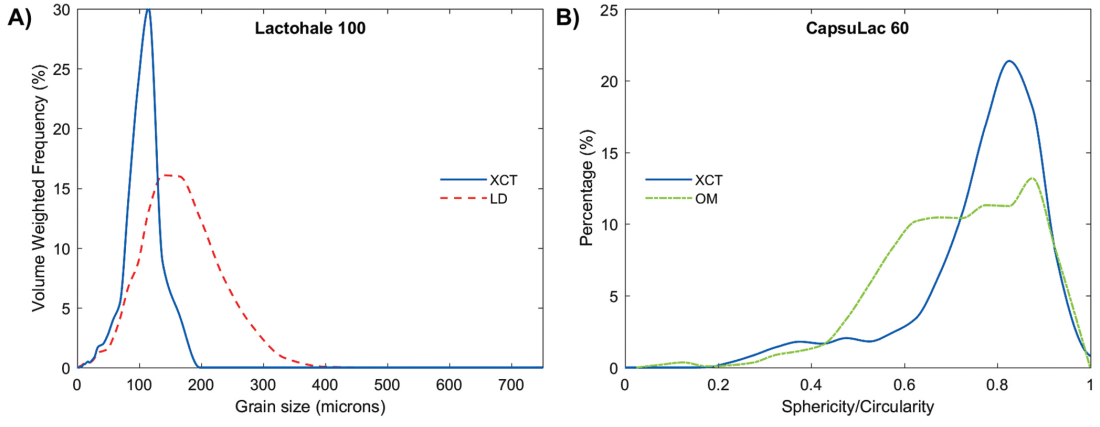

Figure 5. **A comparison between A) particle size distributions for Lactohale 100 produced using x-ray computed tomography and laser diffraction. B) Sphericity/circularity distributions for CapsuLac 60 measured using x-ray computed tomography and optical microscopy.**

Figure 5A compares the size distributions for Lactohale 100 characterized using XCT and LD. The XCT distribution has a lower mode than LD, which is considered to be due to the fact that XCT does not make any assumptions about the particle shape, whereas LD assumes the particles are spherical. The sphericity distributions produced through XCT and OM are shown in Figure 5B for CapsuLac 60: the XCT describes a narrow single modal distribution, while the OM distribution is broad which is considered to be due to the differences when imaging different particle orientations.

XCT can produce more accurate mesoscale distributions than existing techniques, but additionally, because XCT is intrinsically non-destructive, this offers the potential for mesoscale analysis of the powders within a capsule. In addition, exporting the shapes of individual particles within a powder bulk can provide valuable input for simulations to assess the impact of the initial particle packing and orientation on fluidization [38]. This is one of the avenues for future work.

NANO-CT CHARACTERIZATION

The presence of intrinsic lactose fines and agglomerates brings additional complexity to inhalation lactose grades compared to tableting grades. This can be observed in the SEM image of Lactohale 100 [DFE Pharma, Germany] in Figure 6A, where intrinsic lactose fines can be seen on the surface of coarser crystals. Similarly, Figure 6B shows an SEM image of dispersed agglomerates from a Lactohale 300 sample [DFE Pharma, Germany]. But these 2D SEM images produced following dispersion leave several questions unanswered: 1) Do the position of the intrinsic and agglomerated fines relate to the surface properties of the coarse carriers? 2) What is the real microstructure of agglomerated fines within the blend? The recent development of laboratory nano-CT [39] has the potential to unlock the answers. Nano-CT can be used to provide high resolution, 3D images of single lactose crystals, such as the single Lactohale 100 crystal shown in Figure 6C. From this it is, for example, possible to calculate the surface roughness, an important parameter for understanding inter-particle forces and the binding of fines and API to coarse carriers. It should be noted that although Figure 6C shows one single orientation, the virtual volume from the nano-CT scan can be viewed and analyzed in 3D. Nano-CT is also ideal for imaging agglomerated particles, such as Lactohale 300 shown in Figure 6D, providing very detailed structural information.

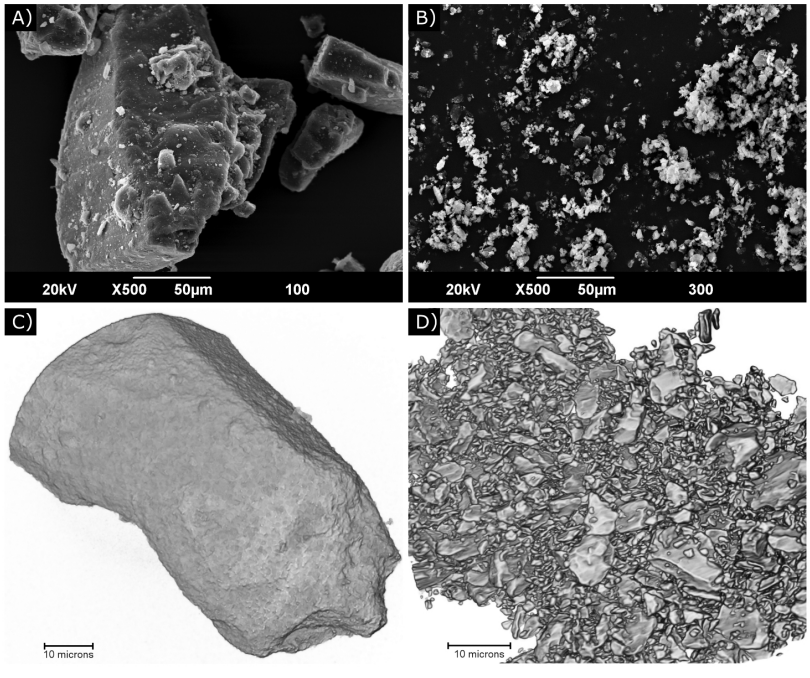

Figure 6. **SEM images showing the particle morphology of A) Lactohale 100 and B) Lactohale 300. 3D visualizations of nano-CT scans of C) a single Lactohale 100 particle, and D) a single Lactohale 300 agglomerate.**

Correlative approaches have also been recently developed to link these different length scales together in the same sample [40]. For an inhalation powder, such a spatial correlative approach, may employ medium resolution micro-CT to scan a bulk powder, from which single particles are identified for higher resolution micro-CT or nano-CT. Correlative approaches could also link features in micro-CT and nano-CT through meso-scale assessments. Multiscale imaging facilities with a range of XCT instruments, for example the Henry Moseley X-ray Imaging Facility at the University of Manchester are well placed to apply such multi-scale and correlative techniques to characterize complex inhalation formulations containing blends of excipients and API.

CONCLUSIONS

XCT is a non-destructive, multiscale technique that shows great potential for the characterization of inhalation formulations. Micro-CT can be used to analyze bulk powder samples on micro- and meso- scales: On the micro-scale, individual particles about 100 μm in size can be visually inspected to inform the 3D morphology; on the meso-scale, 100 mm^3 of powder can be summarized with particle size distribution analysis and shape descriptions, that compare with well-established techniques including LD, OM, and SEM. Taking into account the complexity of the samples scanned, nano-CT can also provide invaluable 3D information relating to the microstructure of the powders, such as surface characteristics of single crystals or the internal structure of agglomerates.

The microstructural information provided through XCT complements all of the work packages involved in the INFORM 2020 project. Synthonic engineering is being employed to understand how crystal surface and morphology control affects agglomeration properties, while integrated measurement techniques such as AFM and surface energy analysis are being used to measure the extent and strength of interactions between particles within the imaged agglomerates. By incorporating both microstructural and inter-particulate force information into computer models, the understanding of powder mixing and aerosolization behavior can be effectively integrated to formulate effective inhaled therapies.

ACKNOWLEDGEMENTS

The authors of this paper are all part of the INFORM 2020 Consortium, which is funded through the EPSRC grant EP/N025075/1. We are grateful to consortium partner DFE Pharma and to Meggle for the supply of the raw materials, and we further acknowledge 3M, AstraZeneca, GlaxoSmithKline, and Malvern Panalytical for their membership and support of the INFORM 2020 Consortium. Beamtime was kindly provided by the Henry Moseley X-ray Imaging Facility (HMXIF), which was established through EPSRC Grant Nos. EP/F007906/1, EP/I02249X/1, and EP/F028431/1. HMXIF is one part of the Henry Royce Institute for Advanced Materials, established through EPSRC Grant Nos. EP/R00661X/1, EP/P025498/1, and EP/P025021/1.

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