New perspectives on the microstructure of inhalable formulations through laboratory 3-D X-ray microscopy

Shedding light on pre-aerosolization microstructure of inhaled aerosols

Parmesh Gajjar, PhD[a] and Ioanna Danai Styliari, PhD[b]
[a]University of Manchester
[b]University of Hertfordshire

Introduction

Delivering drugs effectively and efficiently to the lungs is a complex task in which microstructure plays a critical role. Not only do microstructural attributes such as size and shape affect formulation performance through aerodynamic properties and depth into the lungs that particles reach, but microstructure may induce formulation changes during processing and storage [1]. With this in mind, regulatory bodies are looking towards microstructure as a way of proving equivalence between products produced at different sites, in different batches or by different companies (Q3 equivalence) [2].

Ultimately, it is the aerosolized formulation that is inhaled and recently techniques have emerged for probing microstructural equivalence for post-aerosolized material [3, 4]. However, for portable inhaler devices, the performance of post-aerosolized material is a function of the pre-aerosolized formulation and an aerosolization process involving the device and patient. Subtle differences detected in post-aerosolized material may have their origins in the pre-aerosolized formulation and processing choices. Thus, it is extremely important to understand the microstructure of the pre-aerosolized formulation as well.

Current characterization techniques include laser diffraction (to evaluate particle size), light and scanning electron microscopy (size, shape and morphology), the latter of which are sometimes also equipped with Raman spectroscopy (chemistry). All of these techniques involve dispersion of samples in order for them to be analyzed, i.e., disrupting and changing the pre-aerosolized material through an airflow, and therefore do not allow the pre-aerosolized formulation to be examined in its most raw and undisturbed form.

As a result, there are a number of unanswered questions that relate to the pre-aerosolized formulation: (1) What does the blend formulation truly look like? (2) How do different parts of the blend interact with each other? (3) How do we quantify the microstructure? 4) How does the microstructure change due to manufacturing processes or storage? In the INFORM2020 project (see the article sidebar for a description), we have been attempting to shed light on these questions through X-ray microscopy.

What is 3-D X-ray microscopy?

Many people will have heard of X-rays in the medical context, for example, with single chest X-ray images (radiographs) used to diagnose broken rib cage bones or the build-up of fluid/air in or around the lungs. A computerized tomography (CT) scan collects radiographs from different angles and mathematically reconstructs them to form a virtual volume representation that can used, for example, to examine the three-dimensional (3-D) structure of the lungs. CT scans have also become a popular technique in non-medical sciences, with a wide variety of applications growing rapidly. Some common examples include the examination of geological

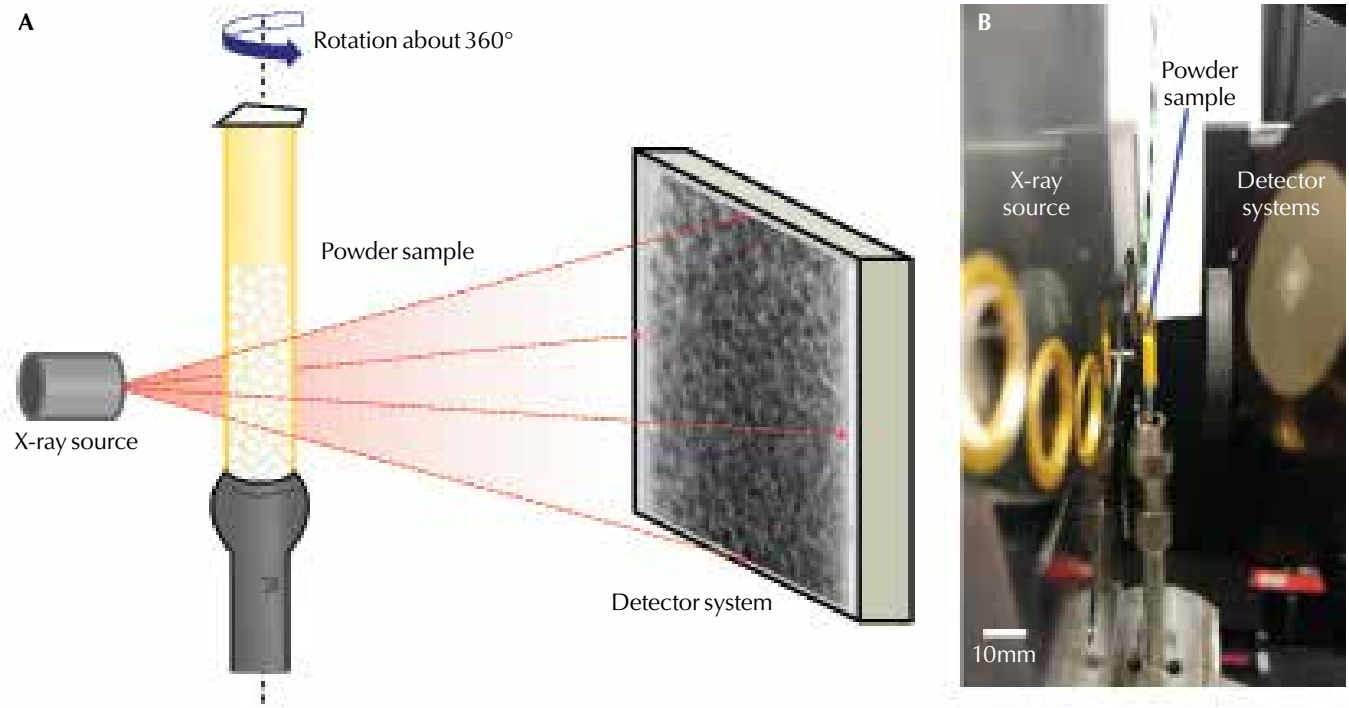
rocks or the structure of polymer composites in materials science. Within pharmaceutical sciences, CT is routinely used to for Quality Check/Quality Assurance (QC/QA) of medical devices, and on the formulation side, is now employed to examine tablets and granules.

The source of X-rays for non-medical CT can either be synchrotron radiation or X-ray tubes. Synchrotron radiation produces very bright X-rays from the motion of electron beams in magnetic fields, but there are only a small number of synchrotron facilities around the world and access can be limited, although some facilities offer mail-in services. X-ray tubes produce lower brightness X-rays but can easily be incorporated into laboratory systems. Such turnkey laboratory CT systems come in a variety of types and sizes, from large drive-in or walk-in systems to benchtop units, with resolutions ranging from meters to several micrometers. The development of X-ray optics has enabled micro- and nano-scale resolution to be achieved, with the similarity to traditional light optics giving rise to the name 3-D X-ray microscopy (XRM). The higher resolutions of XRM systems is particularly important for inhalation formulations where fine particles less than 5 µm are needed to effectively deliver drug to the lungs. Laboratory systems have comparable resolution to synchrotron facilities, but the lower intensity X-ray beam means that scan times are much longer [5].

Laboratory XRM can broadly be split into two categories: (1) micro-scale instruments such as the Zeiss Xradia Versa 520 XRM (Zeiss, Oberkochen, Germany) that can reach a true spatial resolution of 1-1.5 µm and are polychromatic and (2) nano-scale instruments such as the Zeiss Ultra 810 XRM that can reach a resolution of 50 nm. An additional limitation in the latter case is that the field of view is limited to less than 64 µm. A typical micro-scale XRM setup is shown in Figure 1A, with the X-ray source illuminating the powder sample to produce a radiograph on the detector. Notice that the X-rays radiate as a cone and such systems are also known as cone-beam systems. A photograph of the inside of a micro-scale Zeiss Xradia Versa 520 XRM in Figure 1B gives an idea of working distances. Micro-scale XRM instruments typically have a maximum beam energy between 30 kv and 160 kV, and come with in-built software for cone-beam reconstruction (using the Feldkamp-Davis-Kress algorithm [6]) and image artefact correction [7], for instance, beam hardening artefacts arising from the polychromatic X-ray beam. The setup for nano-scale systems is similar, except that the X-rays are parallel, i.e., they do not radiate as a cone, and extra optical elements between the source and sample and between the sample and detector aid in focusing [8]. The Zeiss Ultra 810 XRM instrument also employs a monochromatic X-ray beam with an energy of 5.4 keV.

Figure 1

(A) A sketch of a typical XRM setup, showing the X-ray source, powder sample and detector system; (B) A photograph inside of an Xradia 520 XRM instrument (Zeiss). The scalebar in the bottom left corner gives an indication of the relative sizes and distances. This instrument was used for all of the micro-scale XRM measurements presented in this article.



Sample preparation for XRM is fairly simple, which brings its own advantages for studying inhalation formulations. As shown in Figure 1, powder can be scanned when loosely contained within Kapton tubes [9]. There is no need for glue or fixing agents, coatings or aerosolization, which may disturb the microstructure of a pre-aerosolized formulation. In this way, XRM opens the door to examining pre-aerosolized formulations in their most raw form.

In this article, we explore how XRM can offer new insight into the microstructure of inhalable formulations for dry powder inhalers [9-11]. A typical DPI formulation commonly contains three main components: coarse carrier lactose, micronized lactose and micronized drug. We will show how XRM can provide microstructural information about these components, in isolation and in combination.

3-D size and shape analysis: Carrier lactose

A virtual volume representation of a coarse tableting lactose grade, CapsuLac 60 (Meggle, Wasserburg am Inn, Germany), is shown in Figure 2A. The tomahawk shape of lactose can easily be seen, along with aggregated particles typical of CapsuLac 60. Using advanced image analysis techniques (a modified marker-based watershed algorithm described in detail in reference 10), each individual particle can be identified within the virtual volume, and therefore bulk distributions for size and shape can

Figure 2

(A) 3-D visualization of CapsuLac 60, from XRM data; (B) A comparison of size distributions for CapsuLac 60 from XRM, laser diffraction and optical microscopy; (C) Sphericity/Circularity for CapsuLac 60 as measured by XRM and optical microscopy. Adapted from [7].

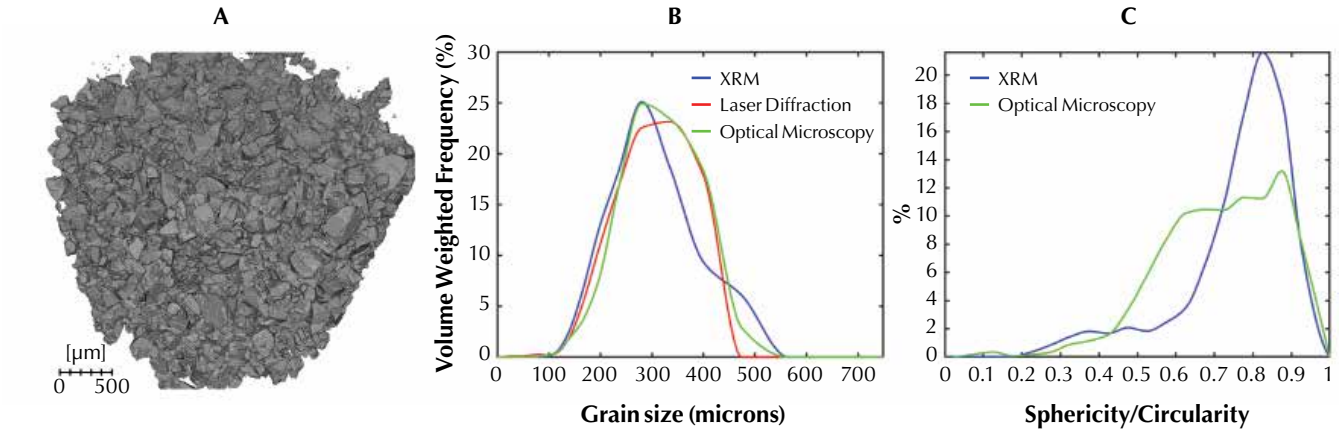
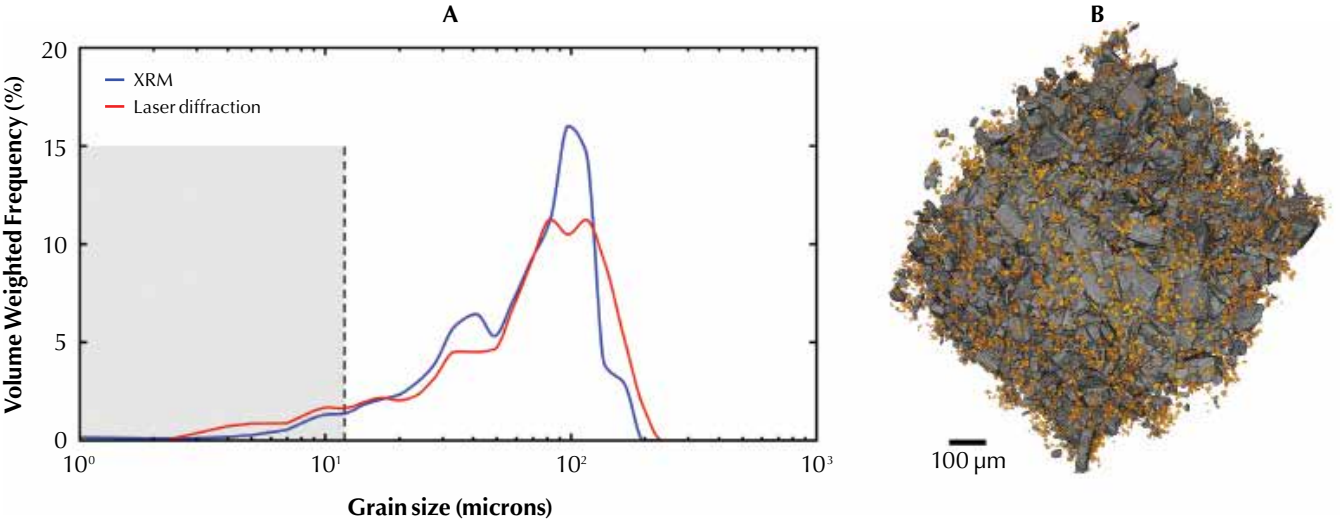


Figure 3

(A) Size distributions for Lactohale 200, measured through XRM and laser diffraction; (B) A 3-D visualization of Lactohale 200, with particles less than 12 µm colored orange. Adapted from [7].



be calculated. As shown in Figure 2B, the size distribution produced from XRM corresponds very well with laser diffraction and optical microscopy, with similar profiles and modal values that are all within 10-20 µm of each other. Calibration against laser diffraction and optical microscopy across various samples gives confidence in the image analysis method and results. In contrast to the size distribution, the sphericity distributions between XRM and optical microscopy in Figure 2C are markedly different: optical microscopy shows a broad, flat distribution with no clear peak, whereas XRM gives a single narrow peak with a normal-like distribution. This discrepancy is due to the difference in two-dimensional (2-D) and three-dimensional imaging, where optical microscopy images each particle from a single direction while XRM uses full volumetric information [10]. The more accurate shape measurement provided by XRM could be used to examine the impact of different processing steps such as sieving and milling on the lactose shape.

Given there are other established methods for particle size analysis, the question arises whether XRM adds anything new? It is encouraging that the size distribution from XRM matches other methods, but in addition XRM brings spatial correlation to the table. The virtual volume gives the size of each particle, as well as its relative position in space, so it is possible to examine interactions between different particle sizes. Consider the size distribution and visualization of Lactohale 200 (DFE Pharma GmbH & Co. KG, Goch, Germany) in Figure 3, with fine particles less than 12 µm highlighted in gray in Figure 3A and colored orange in Figure 3B. Immediately, it can be seen that the fine particle density is not homogenous across Lactohale 200, with some areas showing fewer small particles than others. By sampling different areas of the virtual volume, the number density of fine particles can be quantified as 66458 ± 6033, with a spatial variation of 10% across the sampled regions [10]. Since the fine particles are also more cohesive, this type of analysis may help explain the random chaotic behavior of Lactohale 200 previously observed in fluidization experiments [12]. A further advantage of XRM is that it is non-destructive, and therefore could be used for quality control. For example, in situ size measurements of capsules could provide information on inter- and intra-batch variations.

Nano-structure: Micronized powders

The emergence of nano-scale laboratory XRM instruments (for example, the Zeiss Ultra 800/810 XRM) has helped enable the three-dimensional nano-structure of material to be characterized [9]. Consider the sample of the coarse lactose grade Lactohale 206 (DFE Pharma) shown in Figure 4A, in which it is possible to observe internal voids (cracks) that all lie parallel in one common direction. As can be seen in the 3-D visualization in Figure 4B, the cracks in particle 1 run diagonally upwards, whereas the cracks in particle 2 lie vertical. Previous work has identified the presence of cracks using light microscopy with anise oil as a dispersing liquid, but observations remained very qualitative. The ease with which XRM reveals these nano-structure cracks opens doors to quantitative analysis and comparison between grades, allowing the impact on performance to be accurately assessed.

Nano-structure is particularly important for micronized powders, which are cohesive and readily form agglomerates. Figure 5 shows a sample of micronized lactose grade Lactohale 300 (DFE Pharma) scanned using nano-scale XRM, highlighting the fine granular nature within agglomerates. The porosity can be readily quantified as 72% (i.e., 72% air). Furthermore, it is possible to identify the individual particles within the agglomerate, giving an intra-agglomerate size-distribution in Figure 5D. Interestingly, the distribution is bimodal, with a first peak around 0.5 µm and second at 2.5 µm. The interaction between these different sized particles and their arrangement in 3-D can visualized in Figure 5C, where each particle is colored according to its size.

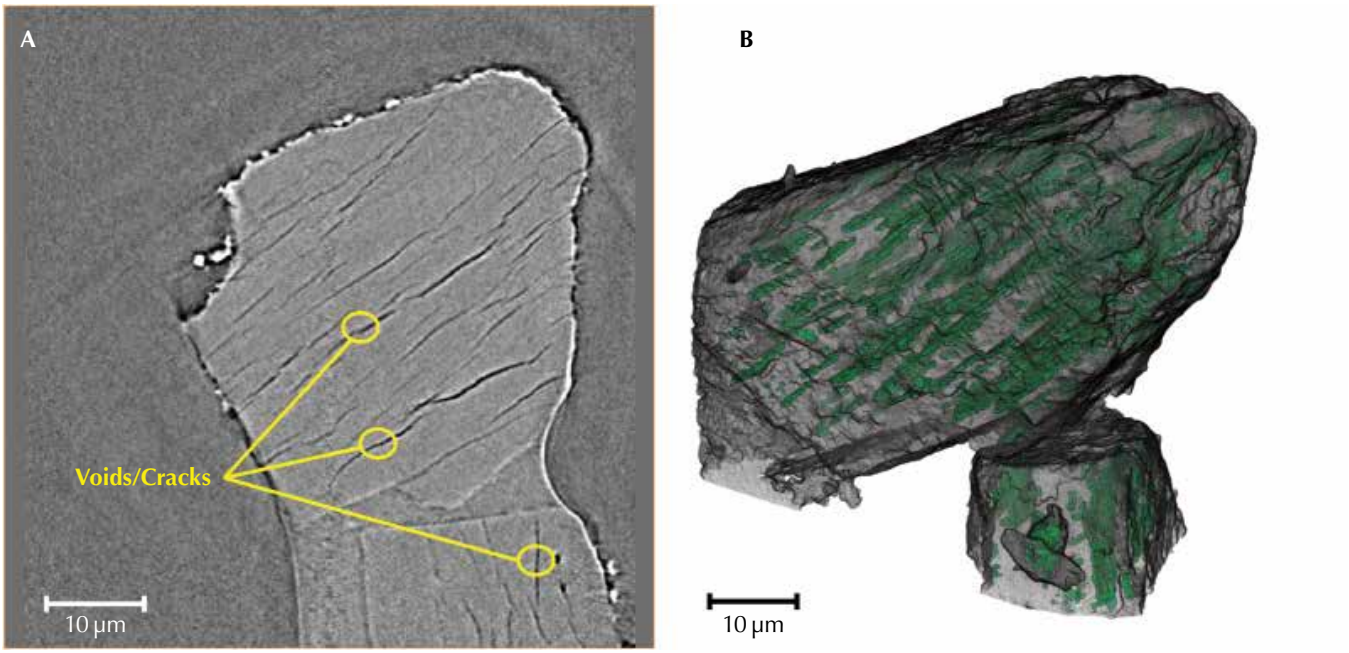
A new way of looking at blends

Spatial interactions between different components of a formulation are important to its microstructure and XRM provides a method for such analysis. Figure 6A shows a virtual 2-D slice through a blend of Lactohale 100 (DFE Pharma) and micronized fluticasone propionate (Coral Drugs, New Delhi, India), with a 10% w/w drug loading. With gray-scale color related to the material density, there are three distinct gray-level values corresponding to air, drug and lactose, with air being the darkest and lactose the lightest in color [13]. Interestingly, the drug coats the lactose in layers. Moreover, the coating is not uniform, with some facets of lactose containing substantially more drug compared to others. This can be clearly seen in the 3-D visualization in Figure 6B. A different type of structure can also be observed in the pink-circled particle in Figure 6A, with several lactose particles bound together, air voids on the inside and the drug confined to the outside of the aggregate structure.

These 3-D images provided by XRM appear even richer when compared to the current standard imaging techniques of using light microscopy or scanning electron microscopy (SEM). Figure 6C shows an SEM image of a similar inhalation blend, in which it is very difficult to tell the difference between lactose and drug. By contrast, the 3-D data provided by XRM allows drug and lactose to be identified, paving the way for quantitative analysis. Work is underway to understand which metrics are important for char-

Figure 4

(A) A virtual 2-D cross-sectional slice through a Lactohale 206 sample, showing the presence of air voids/cracks; (B) A 3-D visualization of the same sample, highlighting the presence of cracks in green. Adapted from [8].



acterizing various drug/carrier blends, along with metrics linked to the performance of each blend. Some XRM systems also give 3-D crystallographic information in addition to pure spatial information. Work is proceeding with these techniques to provide crystallographic insight into the ways different crystal facets affect the drug/carrier binding [14].

What's next?

The desired size range of 1-5 µm for the inhaled API in formulations provides the biggest challenge to any microstructural characterization technique. The encouraging results for DPI formulations discussed in this article open avenues to other inhalable formulations, such as fines-only formulations or spray-dried material. That these results can be achieved on accessible laboratory XRM systems means that wide exploratory work can be conducted, complimenting work at synchrotron facilities where scans can be conducted in much shorter timeframes. This synergy between lab and synchrotron XRM is expected to be particularly powerful for more complex formulations where effects of temperature and humidity on microstructure mean that temporal resolution is critical.

Further work is needed to understand the limits of spatial and chemical resolution, both in image acquisition and image analysis. This will improve our understanding of exactly what can be seen through XRM, what the resulting measurement errors are and therefore where, within the research and manufacturing landscape, the technique will be most suited. By itself, XRM does not offer a one-stop solution, but it certainly provides unique 3-D insight that could powerfully complement other characterization techniques such as Raman spectroscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS).

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Figure 5

(A) A 3-D visualization of a single Lactohale 300 agglomerate from XRM; (B) A virtual cross-sectional slice through the Lactohale 300 agglomerate; (C) A 3-D visualization of Lactohale 300 with constituent particles colored by size; (D) Size distribution for the constituent particles within Lactohale 300. Adapted from [8].

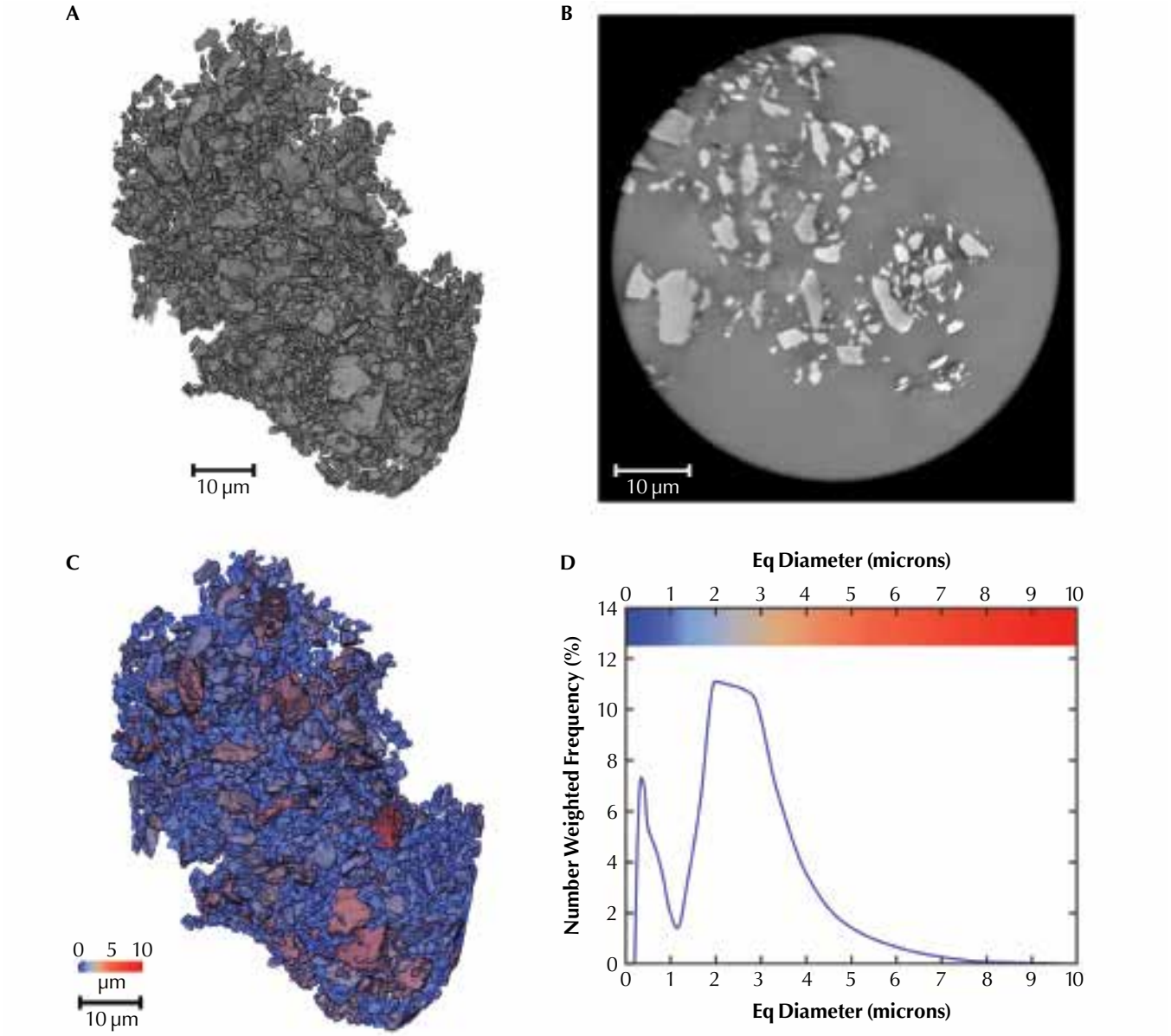

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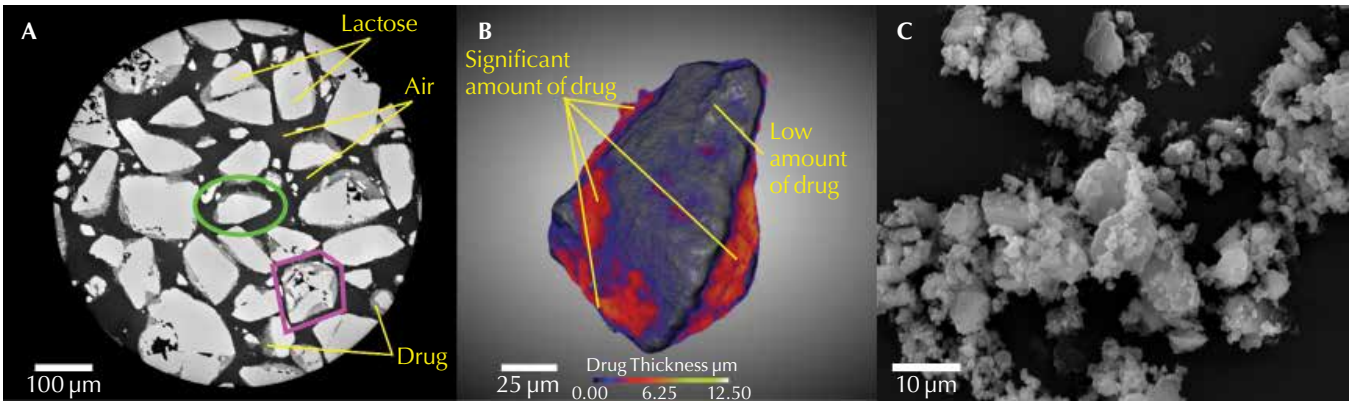
Figure 6

(A) A virtual 2-D cross sectional slice through a 10% w/w blend of fluticasone propionate and Lactohale 100; (B) A 3-D visualization of particle circled in green; (C) An SEM image of a similar blend, with 1500x magnification. Adapted from [8].



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The INFORM2020 Project

The findings discussed in this article are part of INFORM2020, a five-year collaboration of academia and industry to investigate critical parameters that influence the performance of dry powder inhaled (DPI) formulations. In this interview, article authors Parmesh Gajjar and Ioanna Styliari answer several questions about the collaboration.

Inhalation: Who are the INFORM2020 participants?

Ioanna: INFORM2020 is led by Professor Darragh Murnane from the University of Hertfordshire. Academic partners are the University of Cambridge, the University of Leeds and the University of Manchester. Industry partners and contributors are AstraZeneca, GlaxoSmithKline, Kindeva Drug Delivery, Malvern Panalytical and Carl Zeiss Microscopy.

Parmesh: We want to acknowledge the other early career researchers on the project: Dr. Hien Nguyen (Leeds), Dr. Xizhong Chen (Cambridge), Dr. Faiz Mahdi (Leeds) and PhD candidate Vivian Barron (Leeds).

Inhalation: What are INFORM's objectives?

Parmesh: Understanding and controlling the agglomeration phenomenon has been the spark for the INFORM2020 project. As we delved into the project, we realized the material science tetrahedron approach, presented by Sun in 2009 [1], is particularly crucial. Inhaled formulations are a web of complex interrelations among material properties, formulation microstructure, processing and performance. Therefore, we used a synergetic combination of advanced characterization techniques, molecular modeling and computational fluid dynamics to provide new insights and increase fundamental understanding of inhaled medicines.

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Parmesh Gajjar, PhD, is a Postdoc at the University of Manchester, Department of Materials, Oxford Road, Manchester, M13 9PL, UK, parmesh.gajjar@alumni.manchester.ac.uk. Ioanna Danai Styliari, PhD, is a Postdoc at the University of Hertfordshire, Department of Life and Medical Sciences, College Lane, Hatfield, AL10 9AB, UK, i.d.styliari@gmail.com. Darragh Murnane is the lead investigator on INFORM2020 and is a Professor of Pharmaceutics at the University of Hertfordshire, College Lane, Hatfield, AL10 9AB, UK, d.murnane@herts.ac.uk.

Inhalation: Please talk about INFORM's results.

Ioanna: We generated findings in a number of areas (as follows) and have published multiple papers about them:

- three-dimensional (3-D) characterization of inhalation blends, through X-ray Microscopy (XRM)
- predicting particle and surface properties of terbutaline sulphate (an API) and alpha lactose monohydrate (an excipient)
- prediction of agglomeration behavior of blended powder, by molecular modeling that links excipient treatment with physicochemical properties such as surface area and surface energy
- new 3-D flow simulations of the aerosolization process
- modeling pMDI inhaler formulations and fluticasone propionate

Parmesh: We see INFORM2020 as a springboard for future work. The ability to predict properties can facilitate better digital design of inhaled products, while advances in characterization techniques can enable more accurate measurement of physicochemical properties.

Inhalation: Please tell us about your careers to date.

Parmesh: INFORM2020 was my first post-doctoral project. Although the ending of the project is bittersweet, I am very excited to be joining SEDA Pharmaceutical Development Services to study pharmacokinetic and pharmacodynamic modeling.

Ioanna: I am a strong advocate of multidisciplinary approaches to answer pharmaceutical questions, which made being part of the INFORM2020 project so exciting. Next, I will be joining GlaxoSmithKline (GSK) as a materials scientist.

Inhalation: Your new opportunities sound terrific and we wish you the best of success!