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Analytical instrumentation for solid state characterization of inhalable formulations

A review of recent advances and characterization techniques to support respiratory product development

Charina Lampa, Susan Hoe, Dexter D'Sa and David Lechuga-Ballesteros
Pearl Therapeutics, Inc. (an AstraZeneca Company)

Introduction

Modern inhalation drug delivery systems are generally divided into three platforms: pressurized metered dose inhalers (pMDIs), dry powder inhalers (DPIs) and nebulizers. Using each platform to successfully deliver an active pharmaceutical ingredient (API) to the bronchial region requires a narrow range of mass median aerodynamic diameter (MMAD) between 1-3 µm,[1] where the aerodynamic diameter is typically based on primary (or geometric) particle size and particle density. To achieve this requires development of processes whereby the API particle size can be controlled and complex carrier particles that can deliver the API to the desired bronchial regions can be produced. A deep understanding of the critical factors and attributes of these processes and materials can only be obtained through advanced analytical techniques. In this article, advances in analytical instrumentation enabling such particle characterization techniques will be discussed. A summary of techniques and critical quality attributes is shown in Table 1.

Engineering particles within the aforementioned respirable size range, while maintaining favorable bulk material properties, imposes an extra layer of complexity with regard to production and characterization. These engineered particles can either be API, a mixture of formulation excipients or a combination of both. Several well-known processes that have shown success in achieving API particle size control include spray-drying, micronization, and micronization with an additional conditioning step to increase particle crystallinity. Formulation excipients, a major factor in particle engineering, also need to be evaluated for advantages and disadvantages with regard to physicochemical stability and aerosol performance.

Selection of the appropriate excipients and development of API sizing processes necessitate simultaneous improvements in the experimental techniques required to qualify such choices. For instance, processing typically yields amorphous or semi-crystalline material. Amorphous material can be more favorable due to increased bioavailability but can cause several types of physical stability issues. This important attribute needs to be carefully characterized and understood during drug development. Recent advances in Raman spectroscopy, discussed later in this review, could be a favorable alternative or an additional technique to current characterization methods such as x-ray diffraction (XRD) or solid state nuclear magnetic resonance (ssNMR).

Other important characteristics of a material include particle properties such as morphology, surface area and surface energy. These properties have important implications in the aerosol behavior of an inhalable product.[1] Therefore, a thorough understanding and characterization of these properties must be accomplished during development. Scanning electron microscopy (SEM) has been used extensively in industry to observe particle morphology, but recent advances such as nano x-ray computer tomography (NanoXCT) could provide useful complementary information.

For surface characterization techniques, some innovative experimental techniques and approaches have recently been undertaken to enhance current capabilities. For instance, atomic force microscopy (AFM) has typically been used to evaluate surface topology. Recent enhancements in this method include colloidal probe microscopy, which allows for measurement of cohesive forces under several controlled conditions as well as Kelvin probe microscopy, which allows for measurements of surface energy.

For surface area measurements, experiments are typically based on nitrogen adsorption in conjunction with the Brunauer-Emmett-Teller (BET) analysis of the physisorption isotherm. Physisorption isotherm measurements can also be conducted using organic solvent vapors as molecular probes in place of nitrogen to map the chemical nature of the particle surface.

In addition to particle properties, characterization of bulk powder behavior in its native environment is critical for understanding formulation choices and process development parameters. One of the most essential experimental methods to achieve this is the thermal activity monitor (TAM). Recent advances with isothermal calorimetry have allowed for observation of solid

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Table 1

Summary of Techniques and Critical Quality Attributes

Technique	Modifications	Critical Quality Attribute
Raman spectroscopy	Low-frequency shift capability	Solid phase identification and quantification in formulations Crystallinity quantification
Scanning electron microscopy (SEM)	Nano x-ray computer tomography (NanoXCT)	Particle morphology imaging Internal particle structure probing Particle density
Atomic force microscopy (AFM)	Colloid probe microscopy (CPM), Kelvin probe microscopy	Particle morphology Surface topography Surface energy/area Interparticle interactions
Organic vapor sorption and nitrogen adsorption [Brunauer-Emmett-Teller (BET) analysis]	Use of neutral, polar and non-polar molecular probes	Surface area Surface energy
Isothermal calorimetry Thermal activity monitor (TAM)	Perfusion cell TAM capability Pressure-rated TAM ampoule	Reactivity to environmental RH, physical and chemical stability

state changes induced by exposure to various relative humidity (RH) conditions, which is particularly important in predicting long-term stability of a drug product containing an amorphous API or excipients.

Instrumental analysis has made it possible to assess critical quality attributes of formulations and finished products. Such measurements guide the development from feasibility to commercial manufacturing process and ensure the quality of the final product.

Solid phase identification and quantification

Solid phase characterization of API and formulation excipients is crucial to the development of any successful drug product. There are a number of analytical tools available for solid phase characterization yet Raman spectroscopy has been rapidly gaining momentum as a popular technique in both the chemical and pharmaceutical industry. It is a non-destructive analytical technique that requires relatively simple sample preparation. It also offers key information on solid state phases, including but not limited to polymorphism in crystalline materials and stability in amorphous samples. Thorough understanding of these material properties is vital to drug development. Various studies have shown that different solid state phases of excipients and API can have very different performance and therapeutic effects in inhalable formulations.[2-4]

The premise behind Raman spectroscopy is simple: monochromatic light is focused on—and interacts with—a sample. The majority of photons are scattered elastically (i.e., Rayleigh scattering), during which photons are scattered back at the same wavelength as the incident light. However, a small percentage of photons interact with the low-frequency molecular vibrations and polarizable electron cloud density, which causes a shift in the energy of the scattered photons. This is referred to as Raman scattering. This phenomenon forms the basis of Raman spectroscopy. The shift in energy gives valuable information on the inter- and intra-molecular bonds that are present in the sample.

Raman spectroscopy has been demonstrated to be suitable for detection and quantification of small amorphous or crystalline content in the low-frequency region, and identification of subtle molecular conformational changes in the high-frequency region.[5] With traditional dispersive Raman systems, the rejection filters used have a much larger bandwidth, which inadvertently leads to rejection of low-frequency range signals along with the laser line. Most recently, advances in available filter technology have allowed for elucidation into the low-frequency Raman shift region. Please click the link to see Figure 1A, where lattice modes of some common bronchodilators and corticosteroids such as salmeterol xinafoate (SX), formoterol fumarate (FF), glycopyrronium bromide (GP), fluticasone proprionate (FP), mometasone furoate (MF) and salbutamol sulfate (SS) were measured and unambiguously assigned.[6] Differences between crystalline and amorphous GP can also be clearly demonstrated in Figure 1B.[6]

Low-frequency shift Raman spectroscopy has been applied successfully to measurements on single- and multi-component respiratory powders using sample sizes as low as 5 mg.[6] Multi-component powders consisting of a solid matrix of spray-dried, porous lipid particles and some drugs indicated for asthma and COPD have successfully been analyzed on a Raman system capable of accurately measuring in the 15-4,000 cm^{-1} region of the Raman spectra.[7] New advances also allow Raman chemical imaging to be used to determine the solid

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phase of aerosol particles dispersed from a dry powder inhaler, deposited on several different stages of a cascade impactor.[8] For the analysis of non-uniform powder blends such as microcrystalline-lactose blends commonly used in DPIs, a macro-Raman system can be used wherein the optics of the system have been modified to simultaneously measure a sample composition that would be representative of the bulk powder.[9]

A particular area of improvement for existing Raman systems includes investigation of different light sources at an optimal wavelength for particular samples. This could prevent interference from sample fluorescence or sample overheating during fluorescence bleaching.[6] Overall, a high-performance Raman spectrometer with additional ultra-narrow band laser rejection filters could prove to be a valuable investment for use in inhalation drug development.

Particle morphology and density

Knowing the morphology of single particles assists in qualitatively predicting some bulk properties of a powder. Morphology and shape of particles have been shown to impact aerosol performance due to different dynamics during aerosolization and therefore affect deposition in respiratory airways.[10] Scanning electron microscopy (SEM) is a common technique that is routinely used to investigate this property. It affords a close visual of single particles that comprise a bulk powder. Primary particle size is typically a critical quality attribute of most inhalable products and is usually measured by laser diffraction.[11] Algorithms that take the individual particle geometry, rugosity and density are used in particle size measurements.[11] However, SEM can be extremely useful as a complementary technique to visually inspect particle size. In conjunction with energy-dispersive x-ray spectroscopy (EDS or EDX) or even Raman spectroscopy, SEM can be a beneficial tool for semi-quantitatively mapping multi-component systems chemically. This can prove to be important in understanding whether segregation is occurring in a multi-component formulation.[12]

A recent advance in understanding particle morphology, porosity and density is nano x-ray computed tomography (nanoXCT). With minimal sample preparation, this non-destructive imaging technique can be used to create three-dimensional images of a material, including its internal structure. A 3-D rendering of a particle by nanoXCT is achieved by recording ultra-high resolution projections at a controlled range of angles, which are then used to estimate particle size, void-to-particle ratio and density.[13] Some examples of images generated by this technique are shown in Figure 2.[13]

In addition to 3-D visualization, nanoXCT has also been demonstrated to enable quantitative imaging, segmentation and identification of different phases in complex materials at the nanoscale.[14] One of the limitations may be that some internal structures in particles of respirable size approach the 50 nm resolution of the instrument. However, nanoXCT still appears to be a powerful complement to SEM and other microscopy techniques in gaining knowledge of a particle's internal structure, which is useful in predicting aerosol performance critical in inhalable powders.

Particle surface properties (topography, surface energy, surface area)

Surface area and surface energy are critical properties that affect the adhesion forces between particles, between propellant and particle in a pMDI, or device and particle in a DPI. This in turn directly relates to the cohesiveness or dispersability of a powder. Drug particulate adhesion forces have been cited as causing poor aerosol performance in DPIs.[15] This is critical in inhalation therapy, where effective aerosol performance and consistent delivered dose are landmarks of a successful product. Atomic force microscopy (AFM) is a versatile microscopy technique that can be used to obtain surface properties such as topography and surface forces on a single particle scale. It is based upon the principles of a scanning tunneling microscope (STM) and a stylus profilometer.[16] A prefabricated probe with a sharp tip is rastered over a region of a sample of interest. Measuring the vertical and lateral deflections of the probe that occur due to intermolecular forces between the sample and the probe allows a 3-D image of the surface topography to be generated.

One enhancement to AFM is Kelvin probe force microscopy, where the surface potential can be determined by using a conductive AFM probe.[17] Another enhancement is colloidal probe microscopy (CPM), where instead of using a pre-machined sharp tip cantilever, a spherical colloidal particle of interest is attached to a tip-less cantilever with epoxy and used to probe the surface.[18,19]

Preparation of this cantilever can be difficult and time-consuming, but new techniques are emerging that allow for easier and faster preparation. For instance, following a six-step procedure for epoxy and microparticle mounting on custom-designed cantilever holders has allowed for rapid and reproducible production of functional probes.[20] With this technique, porous phospholipid-based particles in some pMDIs[21] have been used to measure microparticle/microparticle adhesion forces under several conditions such as dry, relative-humidity-controlled or suspended in a liquid.[20] Using this data in conjunction with a topographical map can give a 3-D map indicating how adhesion forces vary with particle topography, as shown in Figure 3.[20]

Particles could also be evaluated in model propellant to simulate performance in a pMDI, allowing for quantification of separation energy values.[22,23] Inter-particle forces predictive of flocculation formation, and thus estimation of delivered dose uniformity throughout the life of a pMDI, have also been successfully measured using CPM.[24]

As previously discussed, surface area is an important property in predicting the dispersability of a material, which is a key characteristic of inhalation products. One

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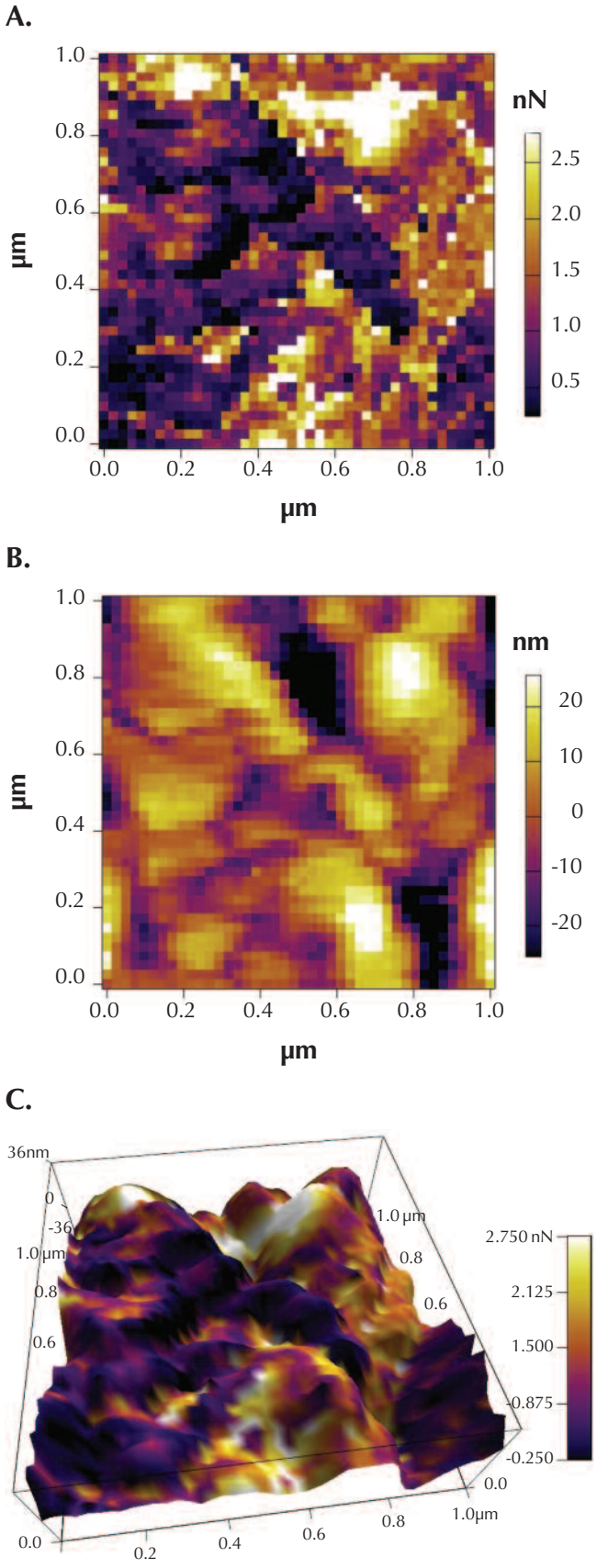
Figure 3

Surface characterization by CPM. (A) Max-pull force map between a phospholipid microparticle substrate and a microparticle colloidal probe; (B) Topographical map of the same microparticle; (C) Combination of (A) and (B) gives a 3-D map of adhesion forces as a function of particle topography.



technique that has been widely used for surface area measurements is nitrogen adsorption combined with Brunauer-Emmett-Teller (BET) physisorption analysis.[25] Microscopy techniques such as AFM and CPM measure surface properties on a single particle level, while using BET analysis (or other models such as the Langmuir model) allows for measurements of these properties on a macro-scale. The bulk material is directly exposed to a molecule with a known cross-sectional area and the number of molecules adsorbed is multiplied by this value to obtain a calculated surface-area measurement. Nitrogen, with a cross sectional area of 0.162 nm^2, is commonly used, but other molecules such as argon, krypton and even octane can also be used.[26]

BET theory assumes that the molecules are closely packed and no surface interactions occur between the molecular probe and the sample surface. However, using molecular probes that have surface interactions with the sample can provide valuable information regarding surface energy of microparticulate systems. In one example, the surface energy of spray-dried inhalable particles was calculated by using acetone and octane as molecular probes instead of nitrogen. This experiment demonstrated that increasing trileucine content of the spray-dried particles decreased the overall surface energy of the material, which correlated to an increase in aerosol performance.[27]

An alternate characterization technique that can be used for surface energy measurements is inverse gas chromatography (IGC). The powder sample of interest is first packed into a column. A series of polar and nonpolar molecular probes are then injected through the column and allowed to interact with the sample to investigate its surface energy. In one example, it was used to positively correlate *in vitro* aerosol performance and surface energy interactions between excipients such as lactose, trehalose dihydrate and mannitol, and active drug material such as albuterol and ipratropium bromide in a DPI.[28] Having a clear understanding of these surface-specific attributes are evidently useful techniques to have in a scientist's toolbox.

In situ solid phase interactions at controlled temperature and humidity in air or in a liquid medium

To get to a controlled respirable size range, most materials have to be processed to reduce the particle size distribution, using processes such as spray-drying or micronization that typically generate amorphous content. Amorphous materials are inherently less stable than their crystalline counterparts and the increased molecular mobility of a material in its amorphous state could inadvertently affect both the physical and chemical stability of the material. Combined with the temperature and residual moisture that material is exposed to during processing and storage, an analytical tool that allows for characterization of the time scale at which long-range molecular motion occurs (i.e., relaxation time) is key to a quick prediction of long-term stability.[27]

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One such tool is a thermal activity monitor (TAM), which is an isothermal microcalorimeter. The addition of an RH-perfusion ampoule to this calorimeter allows for the generation of a moisture-induced thermal activity trace (MITAT). The sample is enclosed in the RH-perfusion cell and exposed to a controlled water vapor pressure at a constant temperature.

The MITAT depicts the thermal response of the pharmaceutical powder as it is exposed at varying relative humidity conditions. In this way, the TAM can be used to determine the stability of the amorphous formulation to relative humidity conditions. The MITAT can be used to determine the onset of moisture-induced physical instability of a material, which can then be used to identify appropriate long-term storage conditions for a product.[1] In Figure 4, the MITAT for sucrose at 25°C is shown.[29]

Another enhancement to the TAM is the use of a pressure-rated ampoule. In pMDIs, a solution or suspension of drug material and excipient is prepared using a hydrofluoroalkane (HFA) propellant as a medium. An analytical tool that could be used to quantify any physical or chemical changes to the material while in the propellant would be a key factor in determining the long-term conditions at which the final product is stable. In the past, a model propellant 2H, 3H-perfluoropentane (HPFP) has been suggested for use in experiments in lieu of other propellants such as HFA-134A and HFC-227, because it has properties similar to HFA propellants but the advantage of being liquid at ambient pressure.[30] In one example, the TAM was successfully used to study the crystallization kinetics of the common inhalable therapeutics beclomethasone dipropionate (BDP) and salbutamol sulphate (SS) in HPFP to simulate physical and chemical changes in the drug products.

Another key extension of TAM capabilities is the modification of an ampoule to tolerate higher pressure, similar to that found in a typical pMDI. For example, the modification of a TAM ampoule to withstand a pressure of 10 bar allowed the investigation of drug stability of formoterol fumarate and beclomethasone dipropionate in HFA-134A.[31] In another experiment, using the same pressure-rated ampoule allowed for rapid determination of compatible formulation excipients, where amorphous glycopyrrolate (GP) and a co-spray-dried combination of GP/DSPC/CaCl2 recrystallized in HFA-134A, but co-spray-dried GP with β-cyclodextrin (βCD) maintained its physical stability[32] as shown in Figure 5.

Undoubtedly, having the capability to perform these measurements is useful in testing and predicting the physicochemical stability of drug material in the propellant as a medium.

Conclusion

The development of some advanced microparticle characterization techniques has been pivotal in facilitating inhalation drug product development activities. For solid phase identification and quantification, low-frequency shift Raman spectroscopy should be considered as a valuable complement or alternative to x-ray diffraction (XRD) or solid state nuclear magnetic resonance (ssNMR). For particle morphology imaging and particle density estimations, nanoXCT is a useful technique to add to the typical scanning electron microscope (SEM). For surface property and interparticle interaction investigations, colloid probe microscopy (CPM), Kelvin probe microscopy and molecular probe adsorption are beneficial tools in addition to atomic force microscopy (AFM) and nitrogen adsorption. Finally, the use of pressure-rated thermal activity monitor (TAM) reaction vessels and perfusion cells are key additions to the usual isothermal microcalorimeter in predicting thermal activity and long-term physical and chemical stability. Using these advanced techniques has allowed scientists to probe attributes that are critical to the quality of inhalation drug product components and support successful development and quality of commercial products.

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Charina Lampa is a Research Associate III, Susan Hoe is a Senior Scientist, Dexter D'Sa is a Scientist and David Lechuga-Ballesteros is a Principal Scientist at Pearl Therapeutics, Inc., (an AstraZeneca Company), 200 Cardinal Way, Redwood City, CA, US, Tel: +1 650 305-2600. Corresponding author: Charina Lampa clampa@pearltherapeutics.com. Website: www.pearltherapeutics.com.

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Figure 1

(A) Raman spectra of various crystalline corticosteroids and bronchodilators in the low-frequency shift (0-200 cm^{-1}) and fingerprint regions; (B) Raman spectra of amorphous and crystalline glycopyrronium bromide (GP).

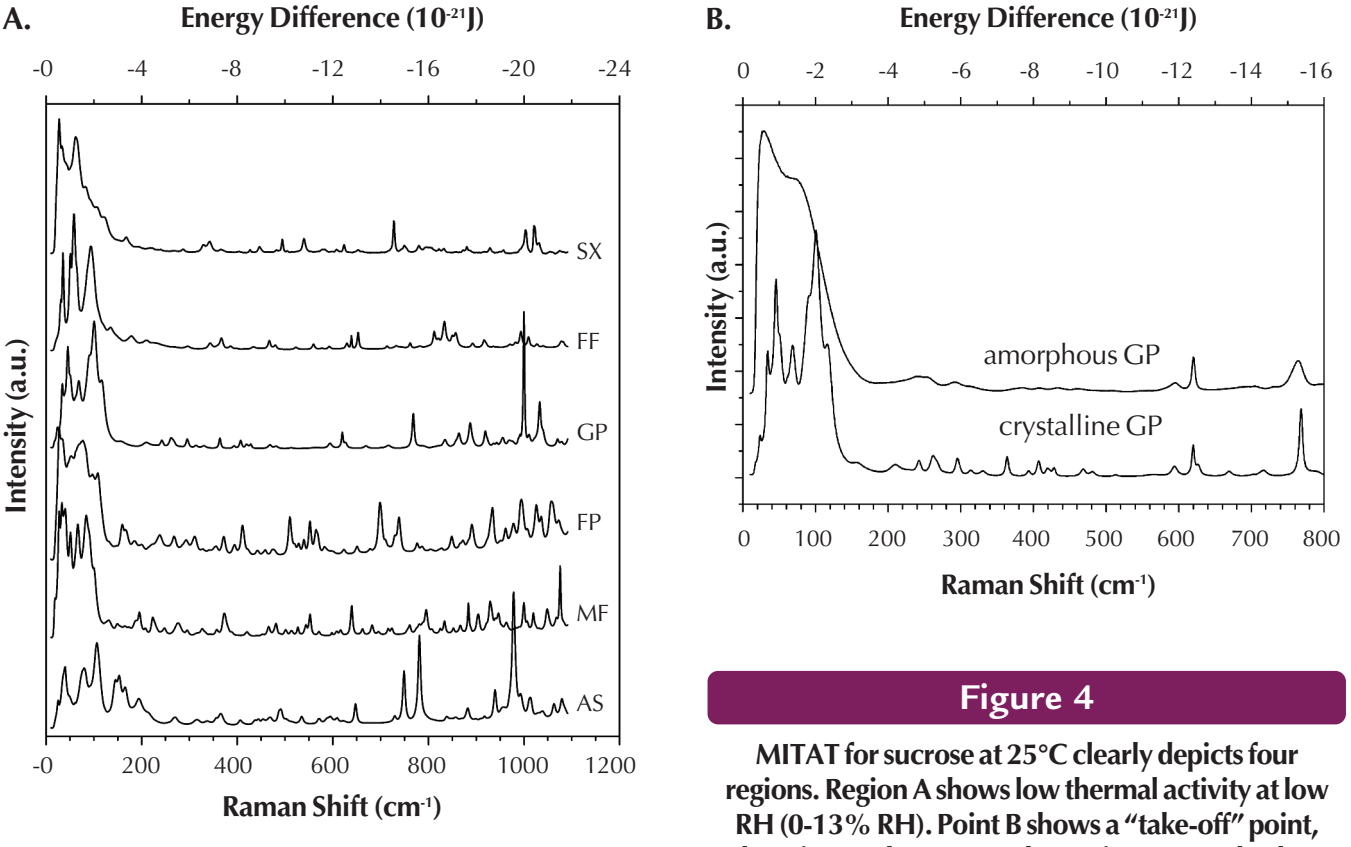

Figure 2

NanoXCT for 3-D imaging and internal structure evaluation. (A) Cross-sectional images of spray-dried mannitol particles; (B) 3-D rendering of spray-dried mannitol particles; (C) Cross-sectional image of a (2-hydroxypropyl)-beta-cyclodextrin (HβCD) particle; (D) 3-D rendering of an HβCD particle.

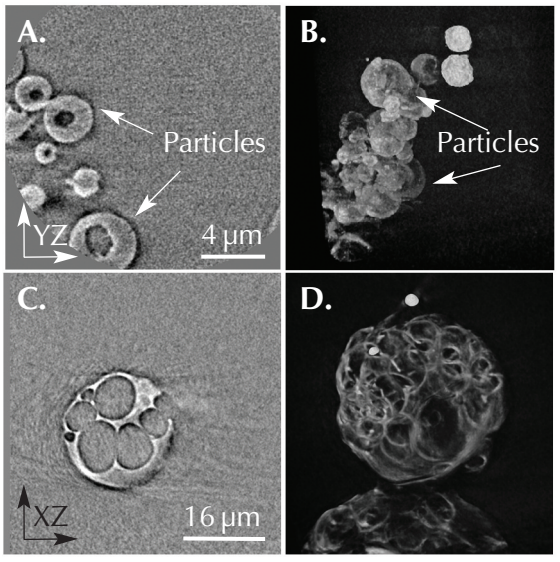

Figure 4

MITAT for sucrose at 25°C clearly depicts four regions. Region A shows low thermal activity at low RH (0-13% RH). Point B shows a "take-off" point, where internal structure change is suspected to have occurred, causing a sudden change in the thermal activity of the material. Region C is an exothermic region correlating with gross structural changes, confirmed with SEM and XRPD. Region D is an endothermic region, where the decrease in activity is suspected to be due to the decreased specific surface area related to structural collapse. From this figure, point B represents a "critical" relative humidity (RH_m) under which long-term stability has been demonstrated.

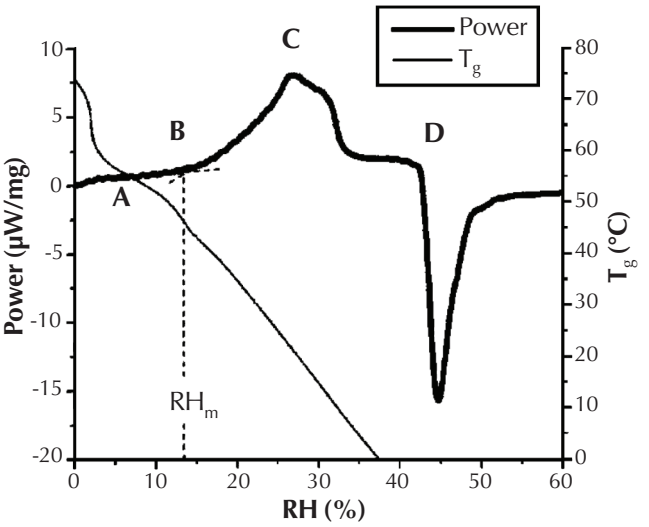

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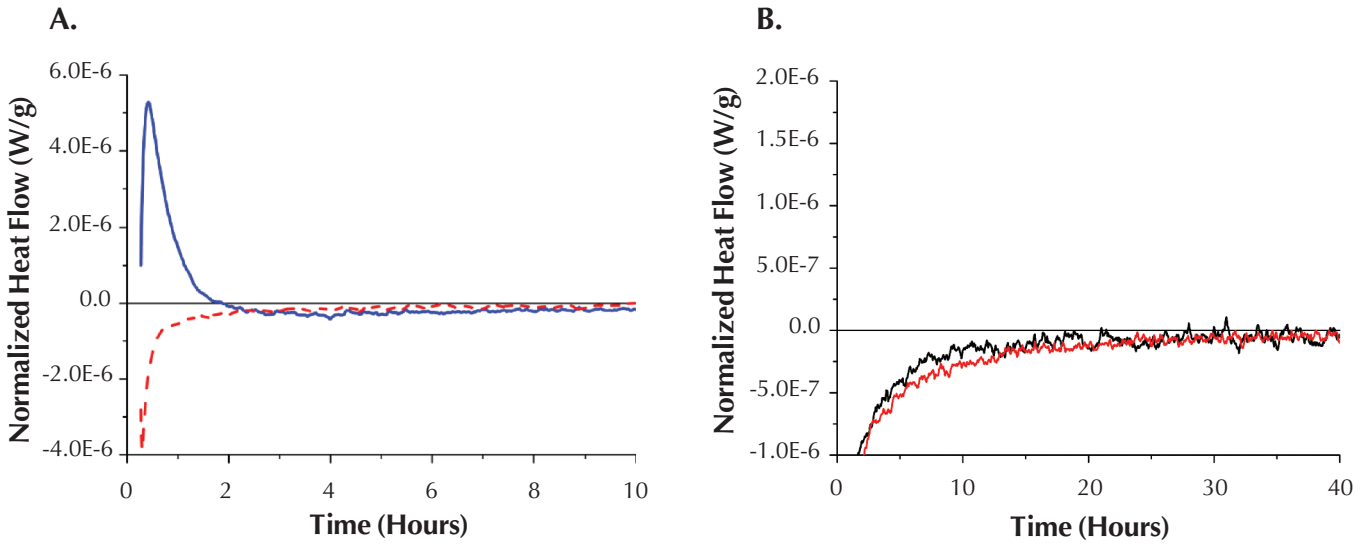
Figure 5

Normalized heat flow signals from a TAM. (A) Stable DSPC/CaCl2 is shown as the red dashed line, while unstable GP/DSPC/CaCl2 is shown as the solid blue line. (B) Stable βCD-only formulation is shown as the black solid line and the stable βCD/GP formulation is shown as the dashed red line.