Solid-state physicochemical characterization and microscopy of particles in dry powder inhalers

A brief review of various important methods

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Inhalable dry powders are generally made up of an active pharmaceutical ingredient (API) i.e., the therapeutic drug. The API can be aerosolized alone as a pure drug aerosol, as an interactive physical mixture of drug and excipient, or as a molecular mixture of drug and excipient. The solid-state characteristics of the inhalable powder are crucial since they affect physical and chemical stability, which in turn affect therapeutic activity and drug bioavailability in the body.

Physicochemical properties such as particle size, particle density, aerodynamic particle size, degree of crystallinity, particle shape, surface morphology, and chemical composition need to be characterized for inhalable solid-state particles that are designed to target select regions of the respiratory tract.[1,2] It is generally well recognized that particles with an aerodynamic size of ≤ 5 µm will effectively reach the smaller airways with high regional deposition and local concentration. Interfacial interactions occurring between adjacent particles can influence aerosol dispersion performance due to non-covalent physical interactions acting on the surface of adjacent particles in the solid state. Therefore, it becomes critically important to comprehensively characterize solid-state physicochemical properties to fundamentally understand the correlative relationship and interplay between molecular properties of the dry powder formulation that can influence the macroscopic properties of aerosol performance, dissolution and *in vivo* therapeutic performance. This fundamental understanding of the correlative relationship and interplay will then enable predictive modeling.

During pharmaceutical powder processing, (e.g., particle size reduction, milling, blending, spray drying and lyophilization), there is intentional or unintentional process-induced phase transformation. Generally, the solid state of a drug molecule is more stable than its liquid counterpart. However, that does not necessarily mean that the solid particles are infinitely stable. Solid particles do undergo several transitions, some of which are noticeable to the eye, such as color change or odor, but more often are unnoticed. These transitions include polymorphic conversion, particle aggregation and decomposition. These factors jointly make it necessary to characterize the drug formulation before and after the processing stage as well as during storage.

Historically, precipitation and crystallization from solution following drying and particle size reduction to an inhalable size range have been carried out to create inhalable powders. More recently, several particle engineering techniques such as spray drying, spray freeze-drying and supercritical fluid technology have been utilized to produce powder for inhalation. These techniques, along with several other pharmaceutical processes such as vapor condensation, supercooling of melt, precipitation from solution, milling or particle size reduction techniques, can induce a certain degree of molecular disorder.[3] The molecularly disordered amorphous state is thermodynamically metastable since it possesses higher Gibbs free energy relative to the thermodynamically stable crystalline state. The amorphous state can be regarded as a supercooled liquid that has lost its ability to flow. With time (i.e., aging), the amorphous state can relax to its thermodynamically stable crystalline state; i.e., enthalpic relaxation. In general, the lower the Gibbs free energy of a polymorph, the lower its apparent solubility and, consequently, its absorption. This can lead to decreased bioavailability of the drug in the body, which in turn can render *in vivo* drug levels below the window necessary for a therapeutic efficacy.

Different crystalline polymorphic forms of a molecule in the solid state influence solubility, stability, bioavailability and processability.[4] Solid-state phase transitions include polymorphic inter-conversions, order-to-disorder phase transitions (e.g., melting from a solid to a liquid) and disorder-to-order phase transitions (e.g., amor-

Table 1

Solid-state characterization methods for particles in dry powder inhalers

Technique	Sample type	Principle/ phenomeneon	Deliverables
DSC	Solid	Thermal	T_{onset} T_{peak} H, C_p
XRPD	Solid	Braggs law diffraction	Diffraction pattern d-spacing
HSM	Solid	Birefringence	Crystallinity, Melting, Boiling
Laser sizing	Dispersed solid	Mie theory	Mean diameter, Span
AFM	Solid	Surface interactions	Surface roughness, Surface energy
SEM	Solid	Elastic/Inelastic scattering	Particle size, Shape, Surface structure
TEM	Solid	Light transmission	Particle internal structure
KFT	Solid	Oxidation/Reduction reaction	Residual water content
IGC	Solid	Interaction with gas	Adsorption isotherm, Glass transition temperature, Surface energy of solids
XPS/EDX	Solid	Electron excitation	Elemental analysis
Raman ATR-FTIR	Solid	Raman effect/IR absorption/Vibrational spectroscopy	Molecular fingerprint

phous-to-crystal transition). Table 1 lists the characterization and microscopy techniques used in characterizing respirable therapeutic powders used in dry powder inhalers (DPIs).

For inhalation powders, the presence of excessive residual water in the solid can potentially render the powder cohesive. This will decrease aerosol dispersion due to increased capillary forces acting on the surfaces of adjacent respirable particles. In addition, the presence of relatively small amounts of residual water in the processed solid, along with a small amount of amorphous character, can influence physicochemical stability and molecular mobility leading to enthalpic relaxation. Consequently, inhalation-grade capsules used in unit-dose DPI products are individually sealed in thick impermeable aluminum foil in plastic trays and multiple dose inhalers have a pre-packed desiccant chamber inside the DPI device to prevent exposure of the inhalable powder to water vapor in the atmosphere. Using these types of packaging strategies and device design, it is possible to maintain reliable performance of the product over a pharmaceutical shelf-life of two years.

Various important physicochemical and microscopic methods are used in the characterization and imaging of respirable particles in the solid state (Table 1). These will be discussed in the following text.

Degree of crystallinity/non-crystallinity determination

Differential scanning calorimetry (DSC) is a thermal analytical technique used to analyze the thermal properties of various types of samples using only a few milligrams of powder. It can be used to detect and quantify phase transitions, phase behavior and molecular interactions. Phase transitions involve molecular reorganization occurring during order-to-disorder and disorder-to-order phase transitions. DSC measures the differential amount of heat flow (energy) absorbed or released from the sample as a function of time and temperature relative to the reference pan. The sample is heated over a temper-

ature range at a fixed scan rate to measure the energy flow with respect to an empty sample holder. Different heating scan rates can be used to detect different types of solid-state phase transitions. For example, to identify a first-order transition such as melting at the melting temperature (T_m), the heating scan rate is generally slow, such as in the range of 1.00-5.00°C/minute. To detect second-order, solid-state phase transitions such as the glass transition temperature (T_g) from the amorphous glass to the rubber phase, faster heating scan rates are required, such as 20°C/minute or 40°C/minute.

Exothermic processes include disorder-to-order phase transitions that include crystallization, recrystallization and decomposition, while endothermic processes include order-to-disorder phase transitions such as melting, dehydration and glass transition. Additionally, DSC can be a useful tool for predicting the extent of miscibility of components in a solid molecular dispersion. For example, an encapsulated or miscible system often exhibits single transition kinetics that may be an average of the two individual transitions, while an immiscible or partially miscible solid system will exhibit two transitions.

X-ray powder diffraction (XRPD) measures the long-range molecular order typical of a repeating crystalline lattice or the absence of long-range molecular order typical of non-crystallinity. X-ray diffraction is based on Braggs law (Equation 1) and provides important information about the presence or absence of long-range molecular order in any given powder sample.

$$2d\sin\theta = n\lambda \quad [1]$$

When an x-ray strikes a crystal surface, part of the beam is scattered by the atoms on the surface while the remaining beam penetrates and strikes the second layer of atoms from which part of the incident beam is scattered and the remaining portion strikes the third layer and so on to form a cone of diffraction. In an orderly arranged crystal, this scattered beam is received from regularly spaced atoms in the crystal lattice which translates to a unique diffraction pattern. Thus, the x-ray diffraction pattern can be thought of as a fingerprint of the material. A highly crystalline compound will exhibit intensive peaks, characteristic of that polymorphic form. Therefore, a change in the diffraction pattern (peaks) in a diffractogram can give useful information about the polymorphic form of the sample. Conversely, an amorphous material exhibits no diffraction peaks due to the lack of long-range molecular order.

During the analysis, the powder sample is filled in the sample holder or a rotating capillary tube. The diffraction is measured from 0-70° 2θ value at various scanning rates. In the majority of the available diffractometers (Figure 1), either the sample remains stationary while the x-ray source and detector move, or the x-ray source and detector are stationary while the sample rotates. In each case, the diffraction pattern generated can then be used to measure the lattice parameter, phase identification and crystal structure.

Figure 1

X-ray powder diffraction instrument

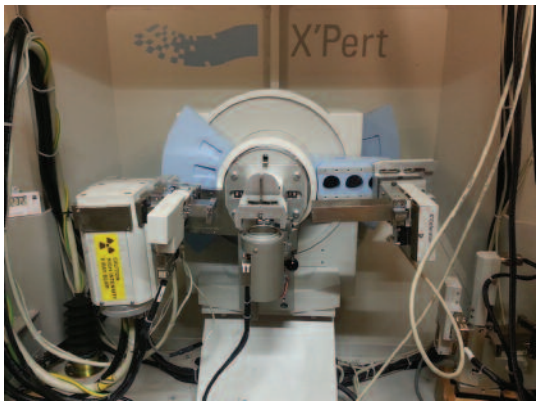
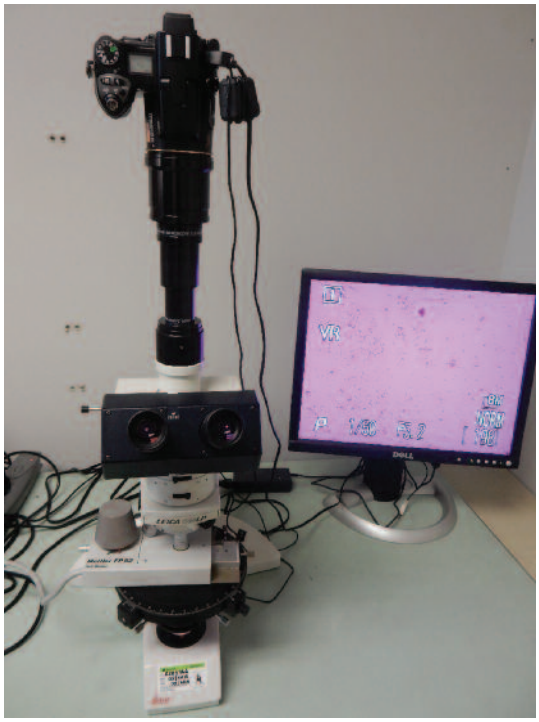


Figure 2

Hot-stage microscope with cross-polarizing lens



Hot-stage microscopy (HSM), also called thermal microscopy, is a combination of thermal and polarized light microscopy techniques used to visualize the sample with temperature. The powder is heated on a hot stage while being visualized under an optical light microscope using a cross-polarizing lens (Figure 2). Light passing through the cross-polarizing lens makes visible the birefringence in anisotropic material, such as a crystal. The presence or absence or change in birefringence that occurs with a change in temperature can then be utilized to characterize the powder sample. The hot stage can also be used with other microscopy techniques, such as scanning electron microscopy (SEM), atomic force microscopy (AFM), Raman and confocal microscopy.

Quantification of residual water content and water vapor/solid interactions

Karl Fisher titration (KFT) is an oxidation/reduction chemical titration where SO_2 is oxidized by I_2. For every mole of water, equimolar I_2 is consumed in the reaction. There are two types of KFT: volumetric and coulometric. Coulometric KFT (Figure 3) is the preferred method due to its low detection limit of 1 ppm-5% w/w. The reaction vessel consists of an alcohol, a base, SO_2 and I_2. For DPI products, it is recommended that the moisture level be kept low as it may affect the particle size distribution, polymorphic form, particle aggregation and possibly, chemical instability. It is important to note that the term "moisture" is not chemically specific for water. Moisture can be measured by a loss on drying (LOD) gravimetric method. The weight loss upon heating can reflect loss of residual water, organic components and/or decomposition of the respirable powder. Residual water content includes both molecularly bound and unbound (free) water. Residual water content in respirable powders is important because it influences aerosol dispersion performance, material handling, powder texture, powder flow, physical and chemical stability of the powder, and can cause particle aggregation. Thus, it is important to be able to accurately determine the residual water content of a powder sample prior to DPI packaging.

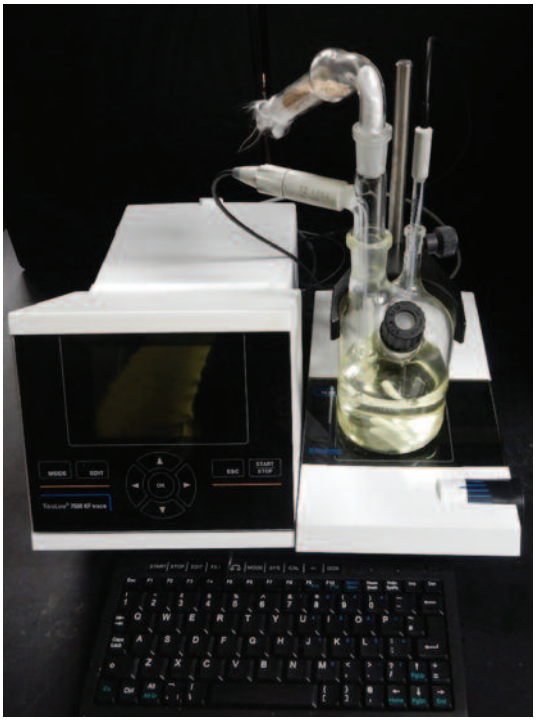
Gravimetric vapor sorption; Sorption is a general term that refers to adsorption (onto a surface), absorption (onto the surface plus into the bulk) and desorption. This technique can be used to quantify minute amounts of amorphous character in the solid (i.e., below the detection limits of DSC and XRPD), the surface area and surface energy of the particles. The isotherm can provide information on porosity, surface area, surface energy and non-stoichiometric vs. stoichiometric water/solid interactions in the context of lyotropic phase behavior. Capillary condensation of water between the solid particles (liquid bridging) can influence "flowability" of the particles. Therefore, it is important to characterize the interaction of water vapor with the solid powder. The change in mass of the sample is measured as a function of relative humidity (RH) and temperature, which is then are plotted as isotherms. The isotherm obtained from this method can been used to complement other techniques like XRPD and DSC in identifying the polymorphic form of the compound.[5,6]

Particle size, shape, distribution and surface morphology visualization and quantification

Laser sizing indirectly measures the particle size distribution of a population of particles where the angle and intensity of the scattered light are used to measure the diameter of an equivalent sphere. The distribution is a weighted, volume-based calculation. This technique is based on Mie scattering theory. A stable and complete dispersion of all particles is required for the measurement. It is necessary to reduce particle aggregation, else multi-modal distribution or disguised (bigger) primary particle size may occur. Commonly used dispersants are water, methanol, ethanol, carbon tetrachloride, chloroform and a surfactant mixture such as span/tween 80 in water. Particle size distribution is critical for DPI formulation, as it influences powder performance properties. Laser sizing is an indirect method of size distribution measurement while microscopic techniques measure particle size directly.

Figure 3

Coulometric Karl Fisher titration instrument



Scanning electron microscopy (SEM); In SEM the electron beam is focused on the sample where elastic or inelastic interaction produces back-scattered (BSE) or secondary electrons (SE). The BSE/SE is captured by the detector to reveal the image of the sample. SEM is a suitable tool to obtain topographic or compositional contrast. SEM has a magnification power of 500,000x; if a field emission gun is used, a resolution of up to 1 nm is possible. SEM has a higher depth of field compared to light microscopy, which gives rise to the appearance of a 3-D image view of the sample (Figure 4). Because inhalation powders are generally sized in the low micron range, SEM is often used to image these particles.

In **transmission electron microscopy (TEM)**, as the name suggests, transmission is the mechanism of interaction between the electrons and the sample of interest. In TEM, the electron penetrates through the entire sample, thus it is capable of showing internal structure such as encapsulation. The electron diffraction pattern of TEM can be used to read phase contrast and crystal structure. TEM uses high-energy electrons in the range of 200 KeV-3 MeV. It has a magnifying power of 1,000,000x with a resolution of up to 0.2 nm. TEM shows a sample at the atomic level and information about the internal structure, while SEM shows external

features such as surface texture, particle shape and size, and topography.[7] These two techniques complement each other in characterizing DPI powders. It should be noted that sample preparation for TEM is time-consuming and is sample destructive.

Surface characterization

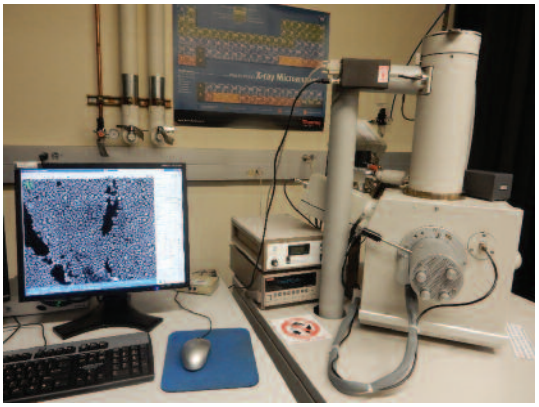
Atomic force microscopy (AFM); Intermolecular forces play a vital role in dry powder inhaler performance, as they influence the aerosolization and dispersion property of the powder formulation.[8] AFM is a surface characterization tool used on thin solid films, solid particles and biological samples. AFM can measure the adhesive/cohesive forces between API particles or API and excipient particles. This technique also measures surface roughness, surface energy and work adhesion, which are critical parameters for DPI formulation. Surface roughness in the particle can potentially lower the particle cohesion. However, increased surface roughness can promote mechanical interlocking that can in turn affect powder fluidization and dispersion. Surface properties important in respirable powders such as surface T_g and surface T_m, surface viscosity, cohesive/adhesive balance and tensile strength can be measured by carefully conducted AFM experiments.

Inverse gas chromatography (IGC) is a surface analytical technique. As the name suggests, it is an inversed gas chromatography where the test sample constitutes the stationary phase and the vapor probe that is injected into the system represents the mobile phase. In standard gas chromatography, a stationary column is used to separate several gasses (mobile phase). In IGC, at constant flow rate, the gas probe molecules are injected through a column packed with powder to determine the surface properties. IGC has been used to identify the influence of milling process on the surface free energy of powder (i.e., polar and dispersive components of surface energy), difference in surface energy of isomers, influence of humidity on surface energy, and to study adhesion properties. It is important to choose the correct vapor probe because a polar probe, when tested with an amorphous material, might end up being adsorbed, which can disrupt surface free-energy calculations. An added advantage is that IGC is non-destructive to the sample.

X-ray photoelectron spectroscopy (XPS) is a surface analytical technique, where the incident x-ray generates a photoelectron from the sample by overcoming the binding energy of an atom. Subsequently, the energy of the photoelectron is measured using electron energy analyzer and electron detectors. The energy of the ejected photoelectron is characteristic of the atom from which it originated. Therefore, this technique is useful in elemental analysis. XPS is only a surface technique, as the photoelectrons released from the depth of the sample have a much lower probability of reaching the detector due to their low energy. From the XPS peak areas, chemical composition of the material can be determined. A chemical shift in the electron can give information about the oxidative state of the sample. The chemical shift is also sensitive to the chemical environment of the atom; i.e., it can reveal information about the type of bonding in which the atom is involved. XPS is a sensitive technique with a detection limit of 0.1-1 atomic percent.

Figure 4

Scanning electron microscope



Chemical characterization and chemical imaging

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) is an infrared spectroscopy technique that gives a molecular fingerprint of the compound. This technique is utilized for structural investigation of organic compounds by virtue of its absorption at the mid-IR range (670-4000 cm^{-1}). It is important to perform ATR-FTIR analysis before and after processing of the powder to verify any structural change, as small changes in the structure and constitution of the compound leads to significant changes in the spectra. In traditional infra-red spectroscopy, the IR radiation is transmitted in the sample and analyzed. However, in ATR-FTIR, the IR beam is passed through a crystal and uses the total internal reflection property of the IR beam when it comes in contact with the sample. The crystal is generally made of diamond, zinc selenide, germanium or silicon. The spectrum obtained is used to determine the functional group of the organic compound by observing the frequency (group frequency) at which IR absorption occurs.

Raman spectroscopy is complementary to IR spectroscopy, as it is a molecular fingerprint based on vibrations of bond stretches not detected by IR. However, some vibrations in a molecule are stronger in Raman than its corresponding IR. Therefore, Raman and IR analyses are complementary. A Raman shift is the principle of this technique. Polarizability and dipole moment of the molecule determine if it is IR active or Raman active. When a sample fluoresces, it can dominate and may obscure Raman peaks, because the Raman signal can be weaker. This can make it challenging to obtain Raman spectra for API if one of the components in the formulation fluoresces. Raman spectroscopy is used in the pharmaceutical industry for quality control.

Confocal Raman microscopy is used for chemical characterization of a sample. It combines the power of a sensitive confocal microscope with Raman spectroscopy. Raman images are then used to obtain information about distribution of a component in the powder sample, composition and identification of material, crystal symmetry, quality of crystals and amount of material present in the sample. An in-depth presentation of Raman chemical imaging has been previously described by the authors.[9]

Energy dispersive x-ray spectroscopy (EDX, EDS, XEDS) is an elemental analysis tool for chemical composition characterization of the powder sample, similar to XPS. When the sample is irradiated with a beam of high-energy electrons or photons (e.g., x-ray), the inner shell electron exits and eventually leaves the atom. An electron from the next outer shell (higher energy) fills this gap. The difference in energy between the two shells may be released as a signature x-ray from the atom since the difference in energy level between two shells is characteristic of the atom. Therefore, this technique can be used for elemental analysis. The downside of this technique is that it is unable to detect elements with atomic number (Z) <11. The detection limit of this technique is also low.

Conclusions

In vitro aerosol dispersion, physicochemical stability, *in vitro* dissolution performance and *in vivo* performance of a DPI formulation are fundamentally influenced by the solid-state interactions, surface properties and physicochemical characteristics of the solid-state particles that comprise an inhalable powder formulation. Particle size, shape, size distribution, surface morphology and interfacial interactions between the solid-state particles in the respirable size range directly impact aerosol deposition in the lungs. With advancements in science and technology in recent decades, an arsenal of state-of-the-art analytical and microscopy techniques is currently available for comprehensive characterization of inhalable dry powders. Several of these state-of-the-art analytical and microscopy techniques are often combined simultaneously *in situ* to limit sample preparation time, save on sample loss and obtain improved results *in situ*. For example, SEM is often combined with EDX, and confocal microscopy is often combined with Raman spectroscopy for chemical imaging (i.e., microspectroscopy). Also, a variable temperature XRPD can be used to understand long-range molecular order properties as a function of temperature. For successful therapy, obtaining reproducible macroscopic performance by controlling the molecular properties in the solid state can reduce variability *in vitro* and *in vivo*. In addition, through comprehensive physicochemical characterization and microscopy, fundamental understanding of the correlative relationship and interplay between the molecular solid-state properties and macroscopic solid-state performance can enable predictive models to be developed.

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Recommended reading

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