Evaluation of Effects of Pharmaceutical Processing on Structural Disorders of Active Pharmaceutical Ingredient Crystals Using Nanoindentation and High-Resolution Total Scattering Pair Distribution Function Analysis

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ABSTRACT: Pharmaceutical unit operations such as milling and compaction can often generate disordered regions in crystals of active pharmaceutical ingredients (APIs). This may lead to changes in a number of important pharmaceutical properties including dissolution, stability, hygroscopicity, and so on. It is therefore important for pharmaceutical industry to evaluate the effects of pharmaceutical processing on API structural orders, and to investigate and develop analytical tools that are capable of accurately detecting and assessing subtle process-induced structural disorders in pharmaceutical crystals. In this study, nanoindentation was first used to determine the intrinsic mechanical properties including hardness and Young's modulus of two API crystals, compounds **1** and **2**. These crystals of different mechanical properties were then milled and compacted under various conditions. The resulting structural disorders in these crystals were subsequently evaluated using synchrotron-based high-resolution total scattering pair distribution function (TS-PDF) analysis. Furthermore, principal component analysis was applied to the PDF data to assess the relative extents of disorders in the API crystals, which showed a good correlation with the process conditions. The study demonstrates that high-resolution TS-PDF analysis coupled with nanoindentation measurement is a valuable and effective tool for detecting and assessing process-induced subtle structural disorders in API crystals. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3879–3890, 2014

Keywords: total scattering; pair distribution function; principal component analysis; compaction; milling; crystal disorder; crystal structure; mechanical properties; nanoindentation

INTRODUCTION

During the manufacturing of drug products containing crystalline active pharmaceutical ingredients (APIs), drug crystals are often subjected to unit operations such as milling and compaction. It is well documented that these pharmaceutical processes can generate various types of defects in API crystals.[1–5] These crystal defects represent regions of higher disorder and higher energy relative to the average overall energy of the crystalline material.[6] These high-energy regions can ultimately affect a number of important pharmaceutical properties of APIs including dissolution rate,[7,8] chemical stability,[9–11] mechanical properties,[12] and moisture sorption.[13] Historically, a range of "bulk" analytical techniques have been used to understand the extent of disorders in pharmaceutical solids including X-ray powder diffraction,[14] density,[15] heat of solution,[16] infrared spectroscopy,[17] dissolution rate,[18] Raman spectroscopy,[19] solid-state nuclear magnetic resonance,[20] dynamic mechanical analysis,[21] differential scanning calorimetry,[22] and water vapor sorption.[2] The disorders generated during milling and compaction are, however, more local in nature, and are not always easily detectable with the aforementioned methods. It is, therefore, important for pharmaceutical industry to investigate and develop analytical methods that are capable of accurately detecting and assessing the extents of subtle changes in API local structures that can be linked with product stability and performance.

In recent years, a powder X-ray diffraction based method known as total scattering (TS) coupled with atomic pair distribution function (PDF) analysis has been utilized to investigate the structural disorders in pharmaceutical crystals.[23–26] Traditionally, powder X-ray diffraction analysis has been focused on the assessment of position and intensity of Bragg reflection peaks to provide information about the long-range order (or the average structure) of a given crystalline phase. The method of TS analysis, on the contrary, uses both the Bragg reflections and diffuse scattering on an equal basis. Assessment of the diffuse scattering component of the powder data provides quantifiable information regarding deviations from the average lattice properties by revealing differences in the local and short-range structure of the material.[27] This structural information is obtained by mathematically treating the TS data to obtain PDFs. The PDF, $G(r)$, provides information on local molecular packing arrangement by giving the number of atoms in a spherical shell of unit thickness at a distance r from any reference atom. Although the atomic PDF analysis of powder diffraction data has commonly been applied in measurements of materials lacking long-range order such as inorganic glasses and liquids,[28,29] with the advance of high-energy X-ray and neutron sources and the availability of increased computing speed, its application in complex pharmaceutical crystalline and amorphous materials has been explored very recently.[30] The quality of TS data is intrinsically related to the wavelength of the X-rays used for data acquisition with shorter wavelengths giving richer information content. For organic materials such as APIs, typical copper

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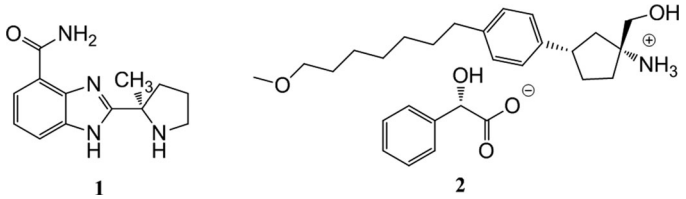
laboratory X-ray radiation source ($\lambda = 1.54056$ Å) results in a PDF of limited real-space resolution.[31] Other important considerations include data acquisition over a wide range of diffraction angles (beyond those typically used in powder X-ray diffraction analysis) and minimized instrument background. An excellent combination of these success factors, often captured by momentum transfer function Q, can be achieved by combining high-energy (synchrotron) X-rays with imaging plate detectors and collecting the data in transmission mode.[32] It has been recommended that PDF data with a Q_{max} of 12.5 $Å^{-1}$ or above should be used for effective fingerprinting of pharmaceutical compounds.[31] Moreover, to utilize TS-PDF analysis to objectively understand the implications in pharmaceutical study, it is critically important to develop and apply sound mathematical and statistical procedures to assess the PDF data.[26,30]

In this work, synchrotron-based TS-PDF analysis with a real-space resolution close to 0.31 Å was applied to characterize the crystal structures of two APIs, compound **1** [2-((*R*)-2-methylpyrrolidin-2-yl)-1H-benzimidazole-4-carboxamide] and compound **2** [((1*R*,3*S*)-1-amino-3-(4-(7-methoxyheptyl) phenyl)cyclopentyl)methanol(*S*)-mandelate], following their milling and compaction under various process conditions. Compound **1** is a PARP inhibitor and compound **2** is an S1P1 antagonist. The chemical structures of the two compounds are depicted in Scheme 1. As characterized by nanoindentation and *in situ* scanning probe microscopy (SPM), compound **1** is a hard, brittle material, whereas compound **2** is a soft, plastic material. TS-PDF analysis revealed that these API crystals of different mechanical properties behaved differently when subjected to milling and compaction. Single-crystal structure analysis and attachment energy calculation were used to explain the different mechanical properties of the two APIs and their milling and compaction behaviors. Furthermore, principal component analysis (PCA) was applied to the PDF data to assess the relative extent of disorders in the API crystals, which showed a good correlation with the different process conditions. The current study demonstrates that high-resolution TS-PDF coupled with PCA and nanoindentation is a valuable and effective tool for detecting and assessing process-induced structural disorders in API crystals.

MATERIALS AND METHODS

Materials

The synthesis of compounds **1** and **2** has been described in earlier publications.[33,34] The Form I anhydrate of compound **1** was recrystallized from water and the anhydrous Form I of compound **2** was recrystallized from isopropanol. The corresponding anhydrous forms of the two compounds were confirmed by standard solid-state characterization techniques.



Scheme 1. Chemical structures of compounds **1** and **2**.

Experimental Section

Nanoindentation and Scanning Probe Microscopy

Nanoindentation and *in situ* SPM imaging of the indent topography were performed using TriboIndenter (TI900; Hysitron, Minneapolis, Minnesota) equipped with a diamond cube corner probe. The probe has included angle of 90° and tip radius of less than 40 nm. The load function for each indent comprised a 5-s loading segment, a 2-s holding segment, and a 5-s unloading segment. Indentations were performed on dominant (103) face of compound **1** and (001) face of compound **2**. Nanoindentation was conducted on at least two freshly prepared single crystals of compounds **1** and **2**, with a total of more than 10 indents between maximum peak load of 200 and 9000 μN. SPM imaging was performed immediately after nanoindentation.

The hardness and reduced modulus of the crystals were determined using the method described by Oliver and Pharr.[35] The hardness, H, is calculated from the indentation peak load, P_{max}, and the contact area, A, following Eq. (1). The contact area is a function of the indent depth, h_c, as shown in Eq. (2), where c_1 to c_5 are fitting parameters. The area function was determined experimentally on a PMMA standard (reduced modulus, E_r, of 5.13 GPa) with 36 indents between 200 and 10,000 μN. The reduced modulus of the crystal was determined from the unloading contact stiffness, S, and projected contact area from the area function using Eq. (3). The Young's modulus, E, was determined using Eq. (4) with a Poisson's ratio, v, of 0.30.

$$H = \frac{P_{max}}{A} \tag{1}$$

$$A = 2.598h_c^2 + c_1h_c + c_2h_c^{1/2} + c_3h_c^{1/4} + c_4h_c^{1/8} + c_5h_c^{1/16} \tag{2}$$

$$E_r = \frac{S}{2}\sqrt{\frac{\pi}{A}} \tag{3}$$

$$E = E_r\left(1 - v^2\right) \tag{4}$$

X-ray Single-Crystal Structure Analysis

Single crystals of compound **1** and compound **2** were individually mounted on MiTeGen polyimide mounts. Intensity data were collected on a Bruker D8 system equipped with an APEXII CD camera. Data were collected at 100 K with graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). Data were collected in four sets using ω–ϕ scans with ω steps of 0.5° and ϕ steps of 90°. A total of 1464 frames were collected with 20 s frame exposures. Data were processed using APEX2.[36] Corrections for Lorentz polarization effects were applied. Absorption was negligible. All structures were solved using direct methods that yielded the nonhydrogen atoms. All nonhydrogen atoms were refined anisotropically. Hydrogen atoms associated with carbon atoms were refined in geometrically constrained riding positions. Hydrogen atoms associated with oxygen atoms were included in the located positions. Refinement was achieved with the use of SHELXTL.[37]