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Crystal face identification by Raman microscopy for assessment of crystal habit of a drug

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ABSTRACT

Crystal habit is one of the key crystallographic characteristics of active pharmaceutical ingredients (APIs), especially those that are poorly soluble. X-ray powder diffraction has commonly been used to assess crystal habit; however, it can only provide macro-information regarding crystal habit for a whole powder sample, not for individual crystals. We describe an approach that uses Raman microscopy for the identification of crystal faces to assess crystal habit at the individual particle level. An antiepileptic agent, phenytoin, was used as the model substance. Phenytoin crystals form a primitive orthorhombic cell. Raman microscopy was used to identify three different patterns of Raman spectra, corresponding to the crystallographic axis that was parallel to the polarization direction of the excitation laser. Thus, a combination of Raman spectra, in which the polarization direction was horizontal and vertical to the morphologically long axis of the crystal, characterized the crystal face. Phenytoin crystals were prepared under various conditions, and the horizontal/vertical combinations of Raman spectra were recorded for individual crystals. The dominantly exposed crystal faces for each condition were identified. This analytical method enables micro-view assessments of crystal habit, which are helpful for identifying the habits of APIs alone and in formulations.

1. Introduction

Crystallographic characteristics such as crystal polymorph, crystallinity, crystal habit, and particle size as well as dispersion of active pharmaceutical ingredients (APIs) and additives in pharmaceutical formulations directly influence their functions, and proper quality control of the formulations requires an accurate and detailed understanding of these properties (Blagden et al., 2007; Erdemir et al., 2007; Llinàs and Goodman, 2008; Lu and Rohani, 2009; Shah et al., 2006). Common analytical methods currently used for this purpose in many pharmaceutical industries include X-ray powder diffraction (XRPD), near-infrared spectroscopy, infrared spectroscopy, and thermal analysis.

Compared with these methods, Raman spectroscopy appears superior in many respects. The advantages of Raman spectroscopy include clear spectral signals, which help to show differences in chemical and physical properties (de Veij et al., 2009; Fini, 2004; Furuyama et al., 2008; Hédoux et al., 2011); these spectra can provide superior quantitative information, especially for the quantification of crystalline/amorphous content (Connolly et al., 2010; Widjaja et al., 2011). Furthermore, high-speed and high-resolution Raman chemical imaging can be performed at single-micrometer resolution applying Raman microscopy (Gordon and McGoverin, 2011; Haaser et al., 2011; Henson and Zhang, 2006; Hubert et al., 2011; Sasić et al., 2005; Sasić and Clark, 2006), which represents an additional advantage over the other methods.

Among the aforementioned crystallographic characteristics of APIs and additives in pharmaceutical formulations, crystal habit is one for which there are very few sufficiently advanced analytical methods available. Although distinct differences in crystal habit between samples can be detected in a macroscopic and qualitative manner on the basis of their diffraction patterns, i.e., changes in signal strength in XRPD (Adhiyaman and Basu, 2006; Kuminek et al., 2013; Nokhodchi et al., 2003), microscopic or quantitative analysis is currently impossible.

More advanced analysis of crystal habit, however, may be feasible using Raman microscopy for which spectral measurement in a single-micrometer wide spot is possible. If there are detectable differences in local Raman spectra among crystal faces and the faces can be identified under a microscope from the spectral

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information, detailed analysis of crystal habit states in micro-areas of an API or tablet cross-section may be possible.

In this study, the feasibility of crystal face identification by Raman microscopy was examined using an antiepileptic agent, phenytoin, as the model substance. The crystal structure of phenytoin had previously been solved (Camerman and Camerman, 1971; Chattopadhyay et al., 1993), and the habit has been characterized by XRPD analysis (Nokhodchi et al., 2003). We followed these crystallographic approaches to analyze phenytoin by single-crystal X-ray diffraction and XRPD and then used Raman microscopy to identify the crystal habit.

2. Materials and methods

2.1. Materials

Phenytoin (5,5-diphenylhydantoin) was purchased from Tokyo Kasei (Tokyo, Japan). Solvents for recrystallization were obtained from standard suppliers.

2.2. X-ray single-crystal structural analysis of phenytoin

Phenytoin was recrystallized from ethanol to obtain a crystal for X-ray single-crystal structural analysis. 0.6 g of phenytoin was dissolved in 20 mL of ethanol at 65 °C. Then, the solution was filtered and cooled to 45 °C at a rate of approximately 5 °C/10 min. After maintaining the solution at 45 °C for 1 h, it was cooled to room temperature at a rate of approximately 5 °C/30 min. A colorless prismatic crystal of phenytoin having approximate dimensions of 0.180 × 0.080 × 0.080 mm was mounted on a glass fiber. All measurements were made on a Rigaku Saturn724 diffractometer (Rigaku Corporation, Tokyo, Japan) using a multi-layer mirror monochromated Mo-Kα radiation. Details of the measurement and structure solution are described in the Supplementary data.

2.3. Recrystallization of phenytoin

Recrystallizations of phenytoin under several conditions were performed to prepare crystals with various habits. Phenytoin was dissolved in organic solvents at 65 °C and filtered. Four crystallization methods from the solutions were performed, as previously described (Nokhodchi et al., 2003).

Method 1: The solution was immediately cooled to −30 °C and left for 2 days.

Method 2: The solution was cooled to room temperature and then kept at 4 °C for 2 days.

Method 3: The solvent was completely evaporated by opening the lid and keeping it at ambient temperature and humidity.

Method 4: A cold poor solvent (water for ethanol, acetone, and acetonitrile; diethyl ether for ethyl acetate and benzyl alcohol) was added to the solution.

The solvent and method used for each sample are listed in Table 1.

Table 1
Sample no. and crystallization conditions of phenytoin.

		Method[a]			
		1 (no.)	2 (no.)	3 (no.)	4 (no.)
Solvent	Ethanol	1–1	1–2	1–3	1–4
	Acetone	2–1	2–2	2–3	2–4
	Acetonitrile	3–1	3–2	3–3	3–4
	Ethyl acetate	4–1	4–2	4–3	4–4
	Benzyl alcohol	5–1	5–2	5–3	5–4

[a] Methods are described in the text.

2.4. X-ray powder diffraction

XRPD spectra were recorded for each recrystallized sample to confirm the crystal forms. The XRPD patterns were acquired on a Rigaku MultiFlex diffractometer (Rigaku Corporation, Tokyo, Japan). The 2θ range was 5–70° at a 0.02° pitch.

2.5. Raman spectral measurement

Raman spectra were recorded on an inVia Raman microscope system (Renishaw plc, Gloucestershire, UK) equipped with a Leica microscope and a 785 nm, 300 mW excitation laser. A 50× objective lens was used and the laser exposure time was 1 s.

3. Results and discussion

3.1. Crystal structure of phenytoin

A microscopic view of phenytoin recrystallized from ethanol is shown in Fig. 1. Structure solution and refinement of the phenytoin crystal were performed as described in the supplementary data. A summary of the crystal data is presented in Table 2.

These crystal data are consistent with those published in literature (Camerman and Camerman, 1971; Chattopadhyay et al., 1993). The Miller indices of the crystal faces and the solved molecular structure of phenytoin are shown in Fig. 2. The solved crystal structure was in good agreement with that published in literature, but it contained partially disordered structures (Fig. 2B). The disordered structures were for the oppositely directed hydrogen bond network on the hydantoin moiety within the crystal (Fig. 3). The structures shown in Fig. 3A and B were found in a ratio of 8:2 in the single crystal. Regardless of the hydrogen bond directional orientation, we proved that the long hydrogen network on the hydantoin moiety was oriented along the *a*-axis, and the hydantoin ring was vertical to the *c*-axis of the unit cell.

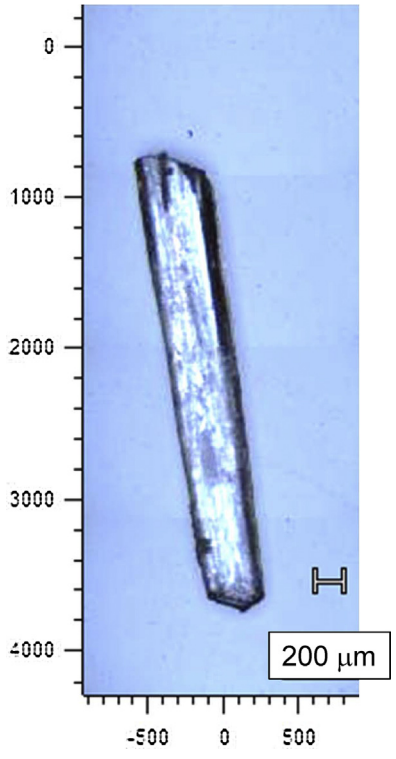

Fig. 1. A microscopic view of phenytoin recrystallized from ethanol.