

Special Feature: Pharmaceutical Analysis (3)

Evaluation of polymorphic forms by powder X-ray diffraction and thermal analysis methods

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1. Introduction

The phenomenon whereby a single substance exhibits multiple different crystal structures is known as crystal polymorphism; the structures are known as polymorphic forms. Many active pharmaceutical ingredients (hereinafter referred to as “API”) of pharmaceutical drugs exhibit polymorphism. Differences in crystal type attributable to hydration may sometimes be referred to as pseudopolymorphism. Each crystal form is referred to as a polymorphic form.

In cases in which an API presents multiple polymorphic forms or pseudopolymorphic forms, the forms must be distinguished and controlled during the development and manufacture of pharmaceutical products, due to the potential for different solubilities and absorption rates. APIs can undergo phase transformations to other polymorphic forms or hydration/dehydration due to external factors such as temperature, humidity, pressure, exposure to light or by the addition of additives. This underscores the importance of assessing early in a research project what conditions may prompt phase transformations and which polymorphic forms may result.

Figure 1 is a flowchart for processes in the Quality Guidelines Q6A stipulated by the International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)*¹. The figure also presents guidelines based on studies investigating the need to set criteria for crystal

polymorphisms of APIs.

As shown in this flow chart, powder X-ray diffraction and differential scanning calorimetry (hereafter abbreviated, respectively, powder XRD and DSC) are fundamental techniques for distinguishing polymorphic forms in APIs. This paper will introduce methods for identifying crystal forms in active pharmaceutical ingredients using powder XRD and DSC, then present a method for examining the polymorphic forms of candidate compounds.

2. Confirming the polymorphic forms of APIs

Each crystal polymorphic form or pseudopolymorphic form exhibits a different crystal structure, each of which exhibit different powder XRD and DSC patterns. We will introduce a method for identifying the particular polymorphic form of the test sample when the polymorphisms for a compound are known.

2.1. Identifying the polymorphic form by DSC

DSC is a technique for detecting variations in thermal energy generated within a sample when the sample is heated or cooled. Since interactive forces such as hydrogen bonds and Van der Waals forces differ for different crystal structures, polymorphic forms of the same substance exhibit different energy states. Where differences in polymorphic form are present, melting points, phase transformation behavior, and the corresponding temperatures often differ significantly. This in turn makes it possible to use DSC measurements to identify crystal forms.

Figure 2 shows a comparison of DSC curves in the heating phase for the commercially available API tolbutamide (a drug used to treat diabetes) and a recrystallized product. In the commercially available API, which we use as a reference, we observe the endothermic peak associated with the phase transformation at 41.7°C. In the test sample, the endothermic peak appears at 102.8°C. These significantly different

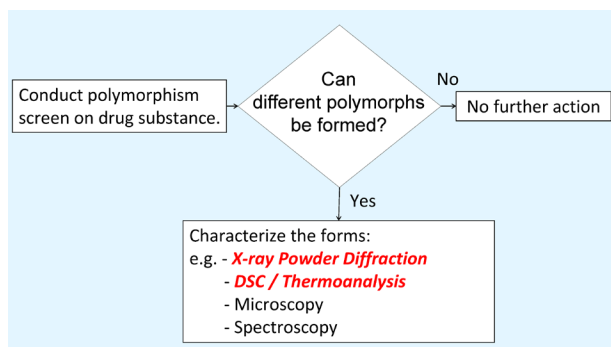


Fig. 1. Investigating the need to test acceptance criteria for polymorphism in drug substances.

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*¹ International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Efforts are currently underway at national, regulatory, and industry levels to achieve international harmonization and meet the needs for consistency generated by the increasingly global process of drug discovery and the inevitable demand for international harmonization, with specialists from Japan, the United States, and Europe meeting to achieve a harmonious set of standards.

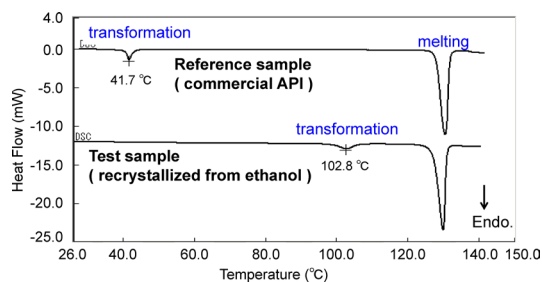


Fig. 2. Comparison of tolbutamide DSC curves.

phase transformation temperatures indicate that the two are polymorphic forms of each other with different crystal structures.

2.2. Identifying polymorphic forms by powder XRD

Given that polymorphisms are known, we can identify the polymorphic forms of a sample by comparing the powder XRD patterns obtained from powder XRD measurements and the standard powder XRD patterns for each polymorphic form. We can obtain standard powder XRD patterns from simulations based on the *d-I* list or the crystal structure extracted from a structure database^{*2}. CIF^{*3} data obtained via single crystal X-ray analysis can also be used for this simulation.

Figure 3 compares the powder XRD pattern of the tolbutamide bulk powder obtained using a benchtop X-ray diffractometer (upper panel) and the standard patterns for tolbutamide of Forms I, II, and III (lower 3 panels). The figure shows that the peak position and relative intensities of the measured data match those of Form I. We conclude that this bulk powder consists of Form I tolbutamide.

In a powder XRD analysis, component concentrations correlate with the ratios of integrated intensities (surface areas) of peaks attributable to the respective components in the sample. Trace components are detected as minor peaks. High intensity data is required to prevent loss of minor peaks within the statistical fluctuations^{*4} observed at the baseline.

Figure 4 shows the profiles of a mixture of Form I and Form II tolbutamide, measured using a MiniFlex600 benchtop X-ray diffractometer. If Form II is a trace polymorphic impurity, this plot shows we can detect Form II in 1 minute, even at ratios below 1 mass%. Note that the measured range corresponds to the region in the

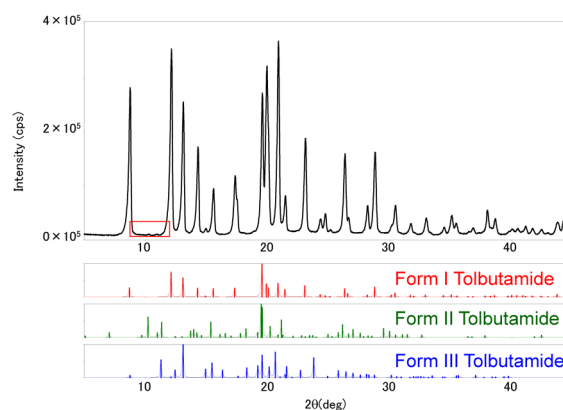


Fig. 3. Results of phase identification of tolbutamide bulk powder (Measurement time: 5 min.).

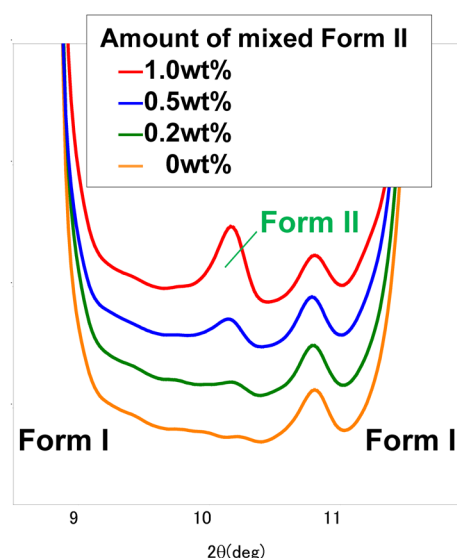


Fig. 4. Detection of trace amounts of Form II tolbutamide from Form I tolbutamide (Measurement time: 1 min. each).

red frame in Fig. 3.

With conventional systems, obtaining this data requires either prolonged measurements on the order of several tens of minutes or powerful X-ray diffractometers equipped with rotating anodes. In contrast, the high-speed, one-dimensional X-ray detectors introduced in recent years allow us to use benchtop powder X-ray diffractometers to confirm polymorphic forms within mere minutes.

2.3. Identifying polymorphic forms of APIs in tablets using powder XRD

APIs are subject to heat, exposure to light, humidity, and pressure during formulation into a product, factors that may result in phase transformations into different polymorphic forms. This creates the need to confirm the polymorphic form of the API in the final drug product. The Bragg-Brentano para-focusing method generally used in optical systems for powder XRD measures powdered samples in reflection geometry.

^{*2} *d-I* list: List consisting of the lattice plane spacing of crystal and relative intensity of peaks.

^{*3} CIF: Data file format developed to facilitate the reading/writing of crystal structure data on computers. The file format was established by members of the IUCr Commission on Journals, Commission on Crystallographic Data, and the Chester office to allow the electronic archival of papers submitted to *Acta Crystallographica*.

^{*4} Statistical fluctuations (in counting): Statistical fluctuations present in X-ray counts. Statistical errors are relatively less significant at higher intensities. Inappropriate measurement conditions and/or low detection sensitivities generally lead to large errors, which in turn make it difficult to detect minor peaks.

But since the tablets must first be ground into powder, phase transformations may still occur during sample preparation, rendering accurate evaluations of the polymorphic form present in the original tablet all but impossible. In addition, when tablets are measured using the reflection geometry without first grinding into powder, X-rays cannot reach inside the tablet. No information can be obtained on the contents of the tablet (Fig. 5). Ideally, we could measure the sample tablets in transmission geometry by a non-destructive method. Conventionally, transmission measurements for such purposes employed parallel beam optics. In recent years, convergent beam optics using an elliptical multilayer mirror⁽¹⁾ offering higher resolution and higher intensity have begun being used.

Theophylline (a bronchial dilating agent) has at least two pseudopolymorphic forms: a monohydrate and an anhydrate. Figure 6 shows XRD profiles for the reference samples theophylline monohydrate (lower panel, in black) and theophylline anhydrate (middle panel, in blue) and the test sample of Theo-Dur tablet (upper panel, in red). The positions of the peak angles marked with solid circles for the test sample match those for theophylline anhydrate, indicating that the tablet contains theophylline anhydrate.

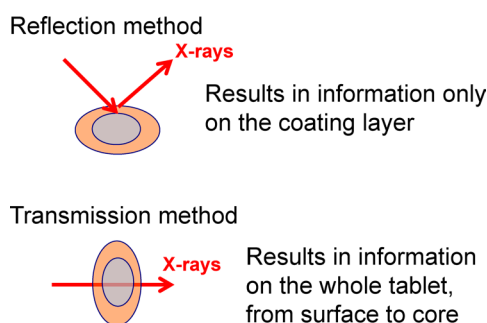


Fig. 5. Differences between reflection and transmission tablet measurement methods.

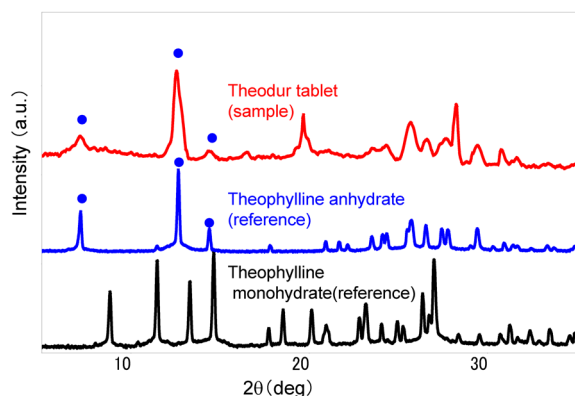


Fig. 6. Comparison of XRD patterns of theophylline monohydrate powder, theophylline anhydrate powder, and the Theo-Dur tablet.

3. Examining the polymorphic form of the candidate compound

APIs are subject to heat and humidity during drug manufacturing and storage. Conventionally, thermal analysis methods are used to assess the conditions at which phase transformation to different polymorphic forms occurs. However, there are cases for which thermal analysis alone cannot distinguish between different polymorphic forms or identify new polymorphic forms (Fig. 7). If DSC measurements identify multiple endothermic/exothermic peaks, for example, the sample will be quenched immediately after the temperature at which the peak appears, in order to further analyze and interpret the thermal reaction. Under certain circumstances, peaks may be overlooked or reactions involving two proximal peaks may be especially difficult to interpret. In such cases, measurements need to be performed repeatedly while increasing/decreasing temperatures; setting the appropriate measurement conditions and data interpretation requires experience. However, if we can simultaneously obtain data on the crystal structure immediately before and after the endothermic reaction during the analysis, such complicated procedures are

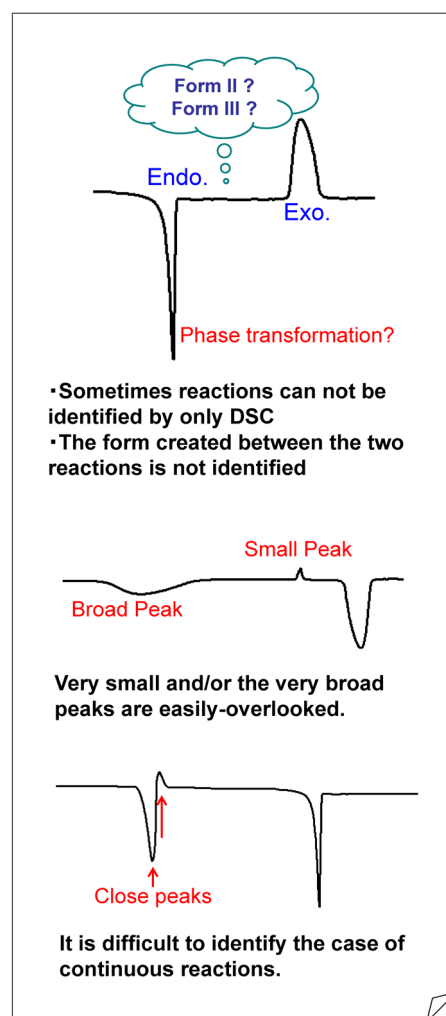


Fig. 7. Problems with non-combined DSC measurement.

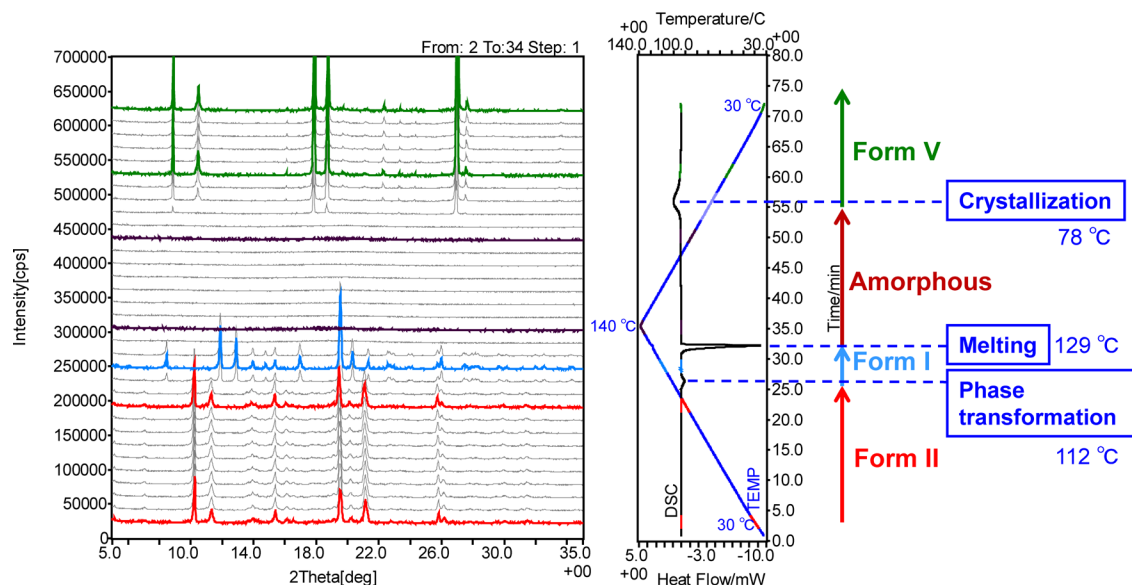


Fig. 8. Results of simultaneous XRD-DSC measurements of Form II tolbutamide.

not required. In response to this need, we developed an instrument called the X-ray DSC that performs X-ray diffraction and differential scanning calorimetry simultaneously (simultaneous XRD-DSC instrument hereafter). This instrument permits control of the analytical atmosphere from low to high humidity.

3.1. Simultaneous XRD-DSC measurement

Figure 8 presents the results of simultaneous XRD-DSC measurements for Form II tolbutamide. The right-hand panel is the temperature and the DSC curve. Time advances from bottom to top. The blue lines in the right panel represent temperature. We see that temperature increases from 30°C to 140°C, then falls from 140°C to 30°C. The black line in the right panel is the DSC curve. The left panel shows the powder XRD patterns measured simultaneously with the DSC measurement in the right panel. Here, we can confirm three peaks in the DSC curve and see corresponding changes in the powder XRD patterns. Changes in powder XRD patterns are due to changes in the crystal structure. Since a change in the crystal structure has been confirmed before and after the first endothermic reaction, we can conclude that this endothermic peak is attributable to a phase transformation. After the second endothermic reaction, the peak corresponding to this crystal structure disappears. Thus, we conclude that melting causes the second endothermic reaction. Subsequent temperature decreases result in the confirmation of a different crystal structure following an exothermic reaction, which can be attributable to crystallization. In this way, this instrument allows interpretation of a thermal reaction based on crystal structure information preceding and following the appearance of a DSC peak. This is the instrument's key feature. If we already know the crystal structure observed in the simultaneous XRD-DSC measurement, we can make a positive identification according to the

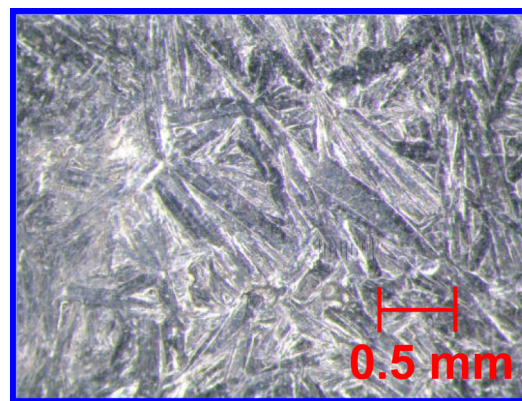


Fig. 9. Photograph of sample after XRD-DSC measurement (tolbutamide).

procedures introduced in Section 2.2.

If the crystal structure observed in the simultaneous XRD-DSC measurement is unknown, we have two ways to analyze the structure. The first is to perform single crystal X-ray analysis; the second is to perform ab initio crystal structure analysis based on powder diffraction data.

3.2. Results of simultaneous XRD-DSC measurement and single-crystal X-ray diffraction analysis

Conventional single-crystal X-ray diffraction analysis requires large single crystals; performing crystal structure analysis using a sample also used for thermal analysis measurements was simply not possible. However, progress in the technologies for high-flux X-ray generators and confocal X-ray mirrors has made it possible to produce high-flux micro beams, and now it is even possible to analyze crystal structures for single crystals at the scale of 2–10 μm . Figure 9 shows a photograph of the sample obtained after the tolbutamide

measurement in Fig. 8. Although the crystal is only $10\mu\text{m}$ thick, we can perform single crystal X-ray analysis and analyze its crystal form as a Form V tolbutamide (Fig. 10).

If the phase transformation temperature is known in advance by the measurements with the simultaneous XRD-DSC instrument, then we can use the single-crystal X-ray diffractometer with a high-temperature gas blower to heat or cool the single crystal directly and maintain the sample at constant temperatures to obtain the data required for structural analysis.

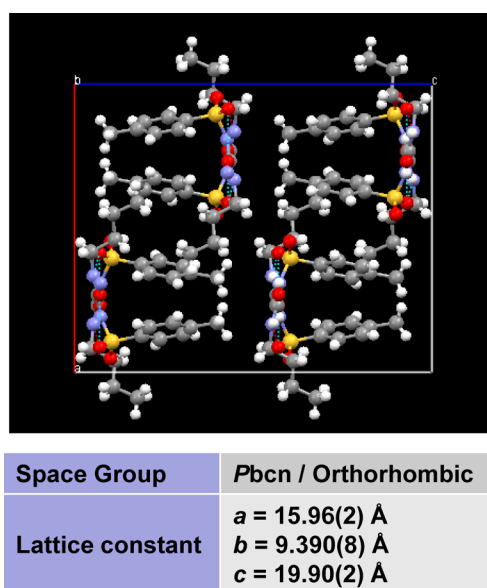


Fig. 10. Results of Form 5 tolbutamide single crystal X-ray analysis.

3.3. Results of simultaneous XRD-DSC measurements and *ab initio* crystal structure analysis from powder diffraction data

For structural analysis of a single crystal maintained at a constant temperature after heating/cooling, we must ensure that no changes have occurred in the external shape of the crystal due to thermal changes. For example, if the sample is a hydrate, dehydration by heating may result in loss of crystal shape. In some cases, it may be impossible to obtain crystals large enough for single-crystal X-ray diffraction analysis. If so, we must use powder diffraction data to determine the unknown crystal structure.

To do this, we determine lattice constants based on the peak position and intensity information; this, in turn, requires high-quality data. Since the reflection method employed for simultaneous XRD-DSC measurement is prone to the problem of preferred orientation^{*5}, a system that allows structural analysis directly from the

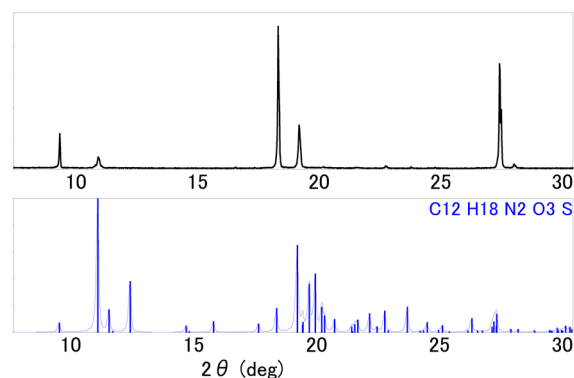


Fig. 11. Powder XRD pattern (upper panel) with preferred orientation and typical pattern (lower panel) for Form 5 tolbutamide.

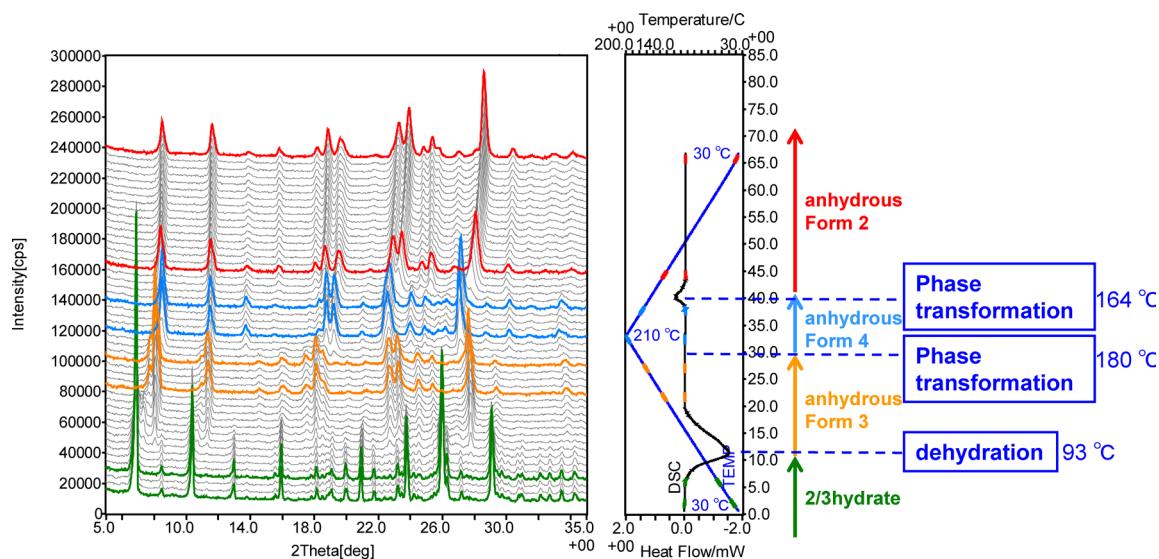


Fig. 12. Results of a simultaneous XRD-DSC measurement of acyclovir 2/3 hydrate. (Sample courtesy of Prof. Katsuhide Terada of Toho University)

^{*5} Preferred orientation: the bias of orientation of a specific lattice plane of a crystal.

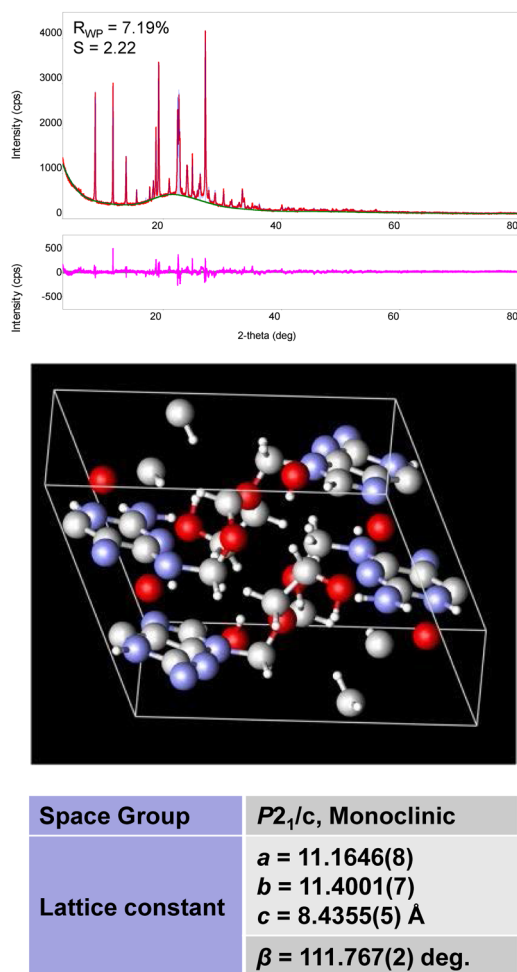


Fig. 13. Structural determination based on powder diffraction data of acyclovir Form 4 (measurement temperature: 210°C).

intensity data of simultaneous XRD-DSC measurements is not feasible. Figure 11 shows a typical pattern for Form V (lower panel) and the powder XRD pattern for Form V tolbutamide obtained in the analysis presented in Fig. 8 (upper panel). We see a difference between peak intensity ratios for the actual measurement and the typical pattern.

For powder samples of unknown structures, we perform measurements by the transmission method while rotating the powder samples inside a capillary to remove the effects of preferred orientation. Structural analysis of crystal phases obtained at high temperature regions, for example, requires performing rotating capillary transmission XRD measurement while heating the sample to above the transformation temperature.

Figure 12 presents the results of simultaneous XRD-DSC measurement for acyclovir (an antiviral agent). Measurements in the heating phase indicate the presence of three other polymorphic forms aside from the initial 2/3 hydrate forms. While the crystal structures for 2/3 hydrate and Form 2 anhydrate obtained at the highest temperature range are known, structural analysis for the Form 3 and Form 4 anhydrides appearing in between had not been performed. We attempted structural

analysis of Form 4 anhydrate using a rotating capillary attachment on a powder XRD diffractometer. Figure 13 presents the results of the powder XRD profile obtained and a structure analysis of the unknown sample using powder XRD. In this way, we determine the crystal structure of Form 4.

3.4. Results of simultaneous XRD-DSC measurement and TG-DTA

As in the case of acyclovir, if the candidate compound is a hydrate, we must determine in advance its dehydration behavior in response to changes in temperature or humidity and predict which hydrate will appear under the conditions. The typical approach in the pharmaceutical industry is to perform equilibrium moisture regain measurements, but such measurements fail to provide information regarding the crystal structure and thus the polymorphic form present.

Figure 14 shows the results of thermogravimetry-differential thermal analysis (TG-DTA) and simultaneous XRD-DSC measurement on nedocromil sodium (abbreviated NS hereafter) trihydrate, an anti-allergenic agent. In a TG-DTA measurement, we change the temperature of the sample and the reference at a constant rate to measure the changes in the weight of a sample (TG) and endothermic/exothermic changes (DTA) simultaneously. Based on the reduction ratio in weight and the temperature at which the reduction occurs, we determine the type of water present in the sample. If the reduction ratio is not stoichiometric, the water is likely to be bulk water; if the ratio is stoichiometric, the water is likely to be lattice water. In the case of NS trihydrate, based on the following calculations, we conclude that the reduction ratio of 7.87% corresponds to the dehydration of the dihydrate:

Molecular weight of NS: 415
 Molecular weight of H_2O : 18
 Molecular weight of $\text{NS} \cdot 3\text{H}_2\text{O}$: 469

Therefore,

we calculate a 7.87% loss in weight as follows:

$$18x/469 = 0.0787$$

$$x \approx 2$$

That is, in the 100–150°C temperature range, we can assume that NS takes the form of monohydrate. On the other hand, the results of simultaneous XRD-DSC measurement shows that the powder XRD patterns in the above temperature range differ from the patterns observed at room temperature. Furthermore, the simultaneous XRD-DSC instrument can be combined with a humidity controller (HUM-SL) to examine the reversibility of the dehydration/hydration reaction by studying the behavior of NS when humidity varies at constant temperature from dry conditions to high humidity up to the maximum humidity of 90%RH

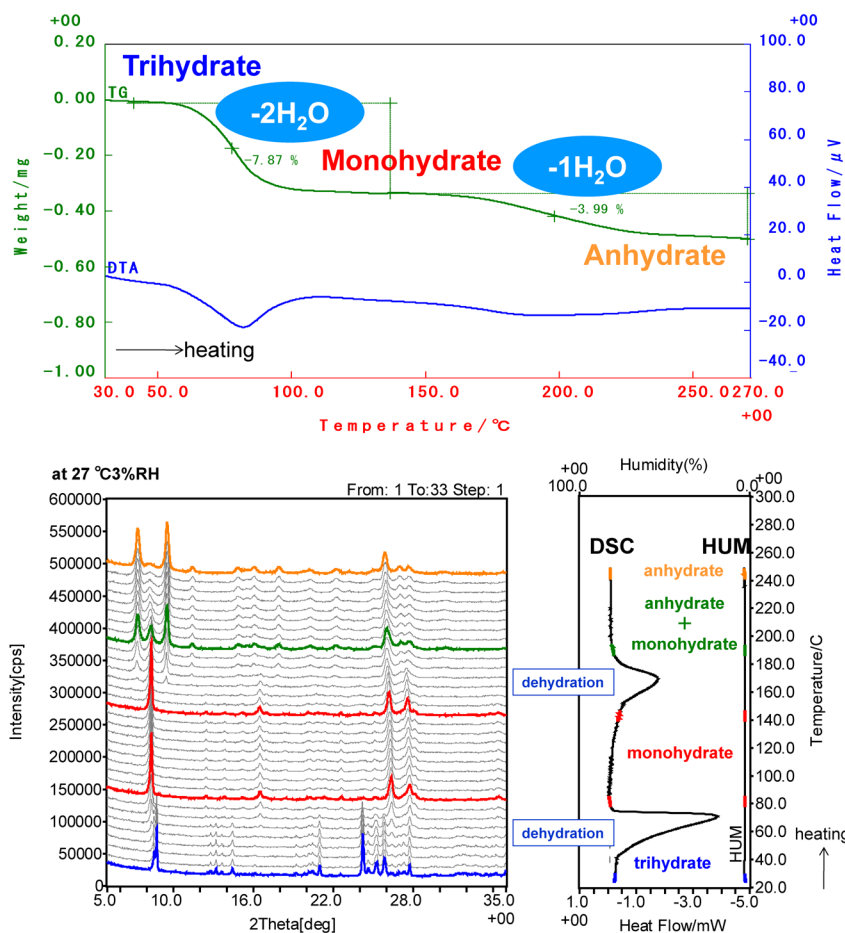


Fig. 14. Results of thermogravimetric differential thermal analysis (upper panel) and results of simultaneous XRD-DSC measurement (lower panel) of NS trihydrate. (Sample courtesy of Prof. Katsuhide Terada of Toho University)

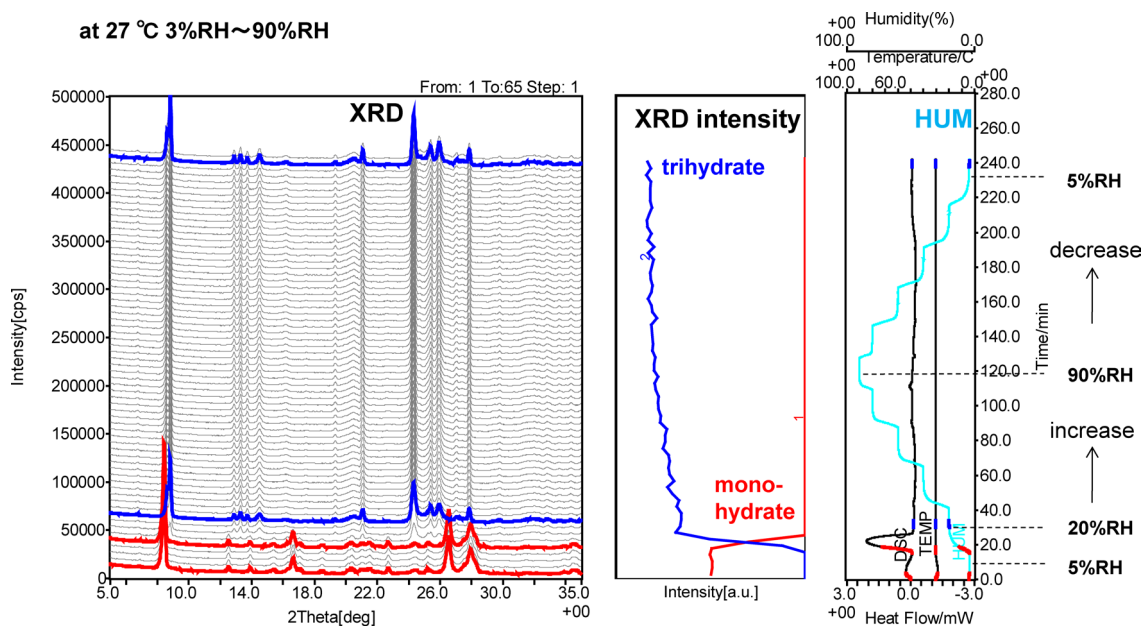


Fig. 15. Results of simultaneous XRD-DSC measurement of NS monohydrate (constant temperature; humidity varied with humidity controller). (Sample courtesy of Prof. Katsuhide Terada of Toho University)

at 60°C (partial water vapor pressure 17.8 kPa) or when temperature varies at constant humidity. Figure 15 shows the results of simultaneous XRD-DSC measurements of NS monohydrate as humidity is increased from 5%RH to 90%RH and decreased from 90%RH to 5%RH at 27°C. The process of NS changing into NS trihydrate through hydration is evident.

4. Conclusions

Powder XRD and DSC are fundamental methods for distinguishing the polymorphic forms of an API. Traditionally, large-scale powder X-ray diffractometers have been used to detect trace polymorphic impurities. Now, high-speed one-dimensional X-ray detectors allow us to perform this analysis with benchtop X-ray diffractometers. Convergent beam optics found in high-end powder X-ray diffractometers allow rapid identification of the polymorphic form in tablets, making it possible to identify the polymorphic form of the API

found in the formulation.

Simultaneous XRD-DSC measurements have been used to investigate the polymorphic form of candidate compounds. Combined use of the system with other analytical methods is now emerging, allowing determination of the structure of new crystal forms or confirmation of the structure of a pseudopolymorphic form having a different hydration number.

Acknowledgments

Professor Katsuhide Terada and Associate Professor Etsuo Yonemochi of Toho University (professor of Hoshi University, now) graciously provided samples and took the time to discuss several issues in fruitful ways. We gratefully acknowledge their support.

Reference

- (1) *The Rigaku Journal (English version)*, **26** (2010), No. 2, 31–32.